

30
21/7/56

EXD.

HÖFCHEN-BRIEFE

BAYER PFLANZEN SCHUTZ - NACHRICHTEN

English edition

8

No. 6, 1955

PUBLISHED BY THE BAYER PLANT PROTECTION DEPARTMENT
LEVERKUSEN AND HÖFCHEN

C O N T E N T S

Page

Dr. Richard W. Wä c k e r s :

Phytophysiological Effect of the Systemic Insecticide
"Systox" (Diethyl thionophosphoric ester of β -oxyethyl
thioethyl ether) 266

Reprints permitted with reference to origin

BAYER LEVERKUSEN (GERMANY)

Department for Plant Protection

Phytophysiological Effect of the Systemic Insecticide "Systox" (Diethyl thionophosphoric ester of β -oxyethyl thioethyl ether)

by Dr. Richard W. Wäckers

CONTENTS

	Page
I. Introduction	266
II. Material and methods	267
III. Experimental section	273
A. Growth measurements	273
1. Testing of the systemic insecticide "Systox" in the cress root test	273
2. Effect of stronger "Systox" concentrations on the growth of the <i>Lemna</i> root	274
3. Increase in the dry weight of suspensions of unicellular algae in the presence of "Systox" and "Schradan"	275
4. Morphological changes of <i>Ankistrodesmus</i> cells in "Systox" solution	277
B. "Systox" concentrations in algae poor in fat and in those which store fat	280
C. Respiration measurements (<i>Ankistrodesmus</i> , <i>Helodea</i> , <i>Tradescantia</i> roots)	284
1. Respiration in "Systox" concentrations of 0.0001 to 0.1 %	284
2. Effect on respiration exerted by several substances related to "Systox"	287
3. CO ₂ evolution in the presence of high "Systox" concentrations	291
4. Reversibility of increase in respiration	291
5. Effect of "Systox" on respiration and synthetic efficiency of <i>Ankistrodesmus</i> and <i>Chlorella</i> in glucose-containing nutrient solution	293
6. Respiratory O ₂ consumption of roots in "Systox" solution	297
D. Measurements of photosynthesis	299
1. Influence exerted by different "Systox" concentrations on photosynthesis	299
2. Reversibility of the photosynthetic disturbances	302
E. Nitrate assimilation (quotients of gas exchange)	305
1. Respiratory quotients in the presence of "Systox" in nitrate nutrient solution	305
2. Measurements of respiratory quotients in nutrient solution with ammonium salt as the source of nitrogen	306
3. Photosynthetic quotients in the presence of "Systox"	307
F. Appendix: Field tests on <i>Vicia faba</i> L.	308
IV. Discussion of the results	313
V. Summary of the results	316
VI. Appendix: Formulae	318
References	319

I. Introduction

The first systemic* pesticides made their appearance on the world market several years ago. For the first time it was possible to control plant pests with practical and useful internal therapeutic* methods.

In contrast to the purely external application used in the past, it is nowadays possible to introduce an insecticidally effective preparation into the vascular system and into the cells of the plants. The great advantages offered by a systemic therapy of this nature need not be discussed in detail in this paper. Reference will be made only to the stability of insecticidal effects produced systemically against weather influences, combined with the long periods the preparation remains effective in the plants, the possibilities of successfully controlling concealed or mining pests, and the harmlessness of the preparation to beneficial insects after it has penetrated into the plant (ecological selectivity).

The introduction of systemic insecticides also brought with it a large number of new problems which in the past had been of little or no significance to phytopathology.

In the previous ectotherapeutic application of pesticides, the plant had in the main only played a passive part as the carrier of the applied substance. With the introduction of systemic preparations, however, it has become an active factor. The plant has gained influence on the absorption of the preparation, on the translocation, the stability and the excretion of the applied substance.

Moreover, the preparation which penetrates into the plant is also able to exert a definite influence on the physiological processes in the plant, to a far greater extent than the substance which remains on the plant surface. Consequently the introduction of the systemically effective pesticides has rendered it necessary to examine the nature, extent and importance of interrelations between the plant and the preparation. As a result a new and close contact has developed between applied botany and phytophysiology.

The first systemic insecticide to appear on the German market in 1952 was "Systox" manufactured by Farbenfabriken Bayer Aktiengesellschaft (Leverkusen). The insecticidal constituent of the preparation is the diethyl thionophosphoric ester of β -oxyethyl thioethyl ether. "Systox" had already been subjected to thorough practical tests both in Germany and abroad over a period of many years under the factory designation "8169" (Ashdown and Corderner, Bosse, Zattler etc.).

Despite the very high standards which have to be fulfilled by a systemic preparation with respect to plant compatibility, it was established in experimental work that the preparation has a really good physiological compatibility with plant cells. It was found to be incompatible only with certain apple varieties (McIntosh, Melba, Guldborg) and the chrysanthemum species *Blanche Poitevine*.

* The term "internal therapy of the plant" was introduced by A. Müller in the middle of the twenties. In 1947, Martin proposed the adoption of the term "systemic" pesticide, which appears to have found acceptance on an ever increasing scale.

In addition to this finding, a large number of publications unanimously point out that the yields, the growing power and the appearance of plants treated with phosphoric esters, in particular with "Systox", are considerably superior to the control plants, also when there is no really serious infestation by pests (Endrigkeit, Kasting and Woodward, Ripper and co-workers 1949, Wolfenbarger, Zattler 1951 and 1952)..

These publications are reports of results obtained in field tests. Therefore, clarity must be obtained as to the possibilities and limits of a field experiment when evaluating such information. The field test is not so much a direct research aid as a means designed mainly for merely checking facts obtained under outdoor conditions. The abundance of influential factors present outdoors mostly renders it impossible to make a clear causal analysis.

This paper is concerned chiefly with the question of the physiological compatibility of "Systox". Studies have been made of the nature of damage observed following excessive dosage and also of any growth regulating effects of the preparation on the treated plants. In order to be able to trace back the problems as far as possible to the fundamental physiological processes, the investigations had to be based in the main upon the laboratory experiment. With the aid of results obtained in this manner, an explanation can be furnished for observations made in the field and for the results obtained in outdoor tests so that under certain circumstances it may be even possible to make a causal analysis.

II. Material and Methods

1. Preparation

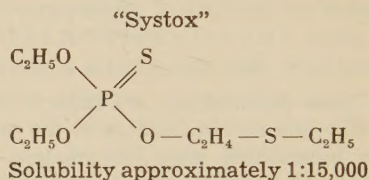
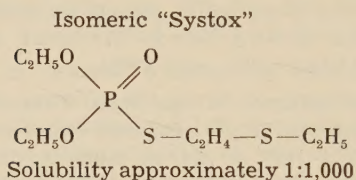
The preparation "Systox" now available on the market is made up of 50 % active substance* and 50 % emulsifier 233.

The emulsifier is a polycyclic compound with a polyoxyethylene chain. In the group $(-O-CH_2-CH_2)_n$, n has a value of approximately 10. Substances of this type possess a smaller wetting power than the ionized wetter of the paraffin chain salt type. On the other hand, they are far more capable of reducing interfacial tensions, for which reason they are particularly suitable as emulsifiers.

The active substance of the commercial preparation consists of a mixture of "Systox" (P=S isomer) and the isomeric "Systox" (P=O isomer), in which the P=O isomer is the main constituent. The physicochemical data for these substances are to be found in the publication of Schrader. A feature of

* The term "Wirkstoff" (active substance) has been in use for more than 25 years to cover vitamins, enzymes and hormones. The application of this term to the phytotherapeutically active constituents of pesticides is, therefore, not a fortunate solution. In particular, it appears illogical to use the term in the phytophysiological formulation of the problem, especially when it is required, on the other hand, that the "active substance" should have no effect on the plant. However, the term has been widely adopted to distinguish these substances from the added inert materials, wetters and emulsifiers. Therefore, it will be used also in this sense in this paper.

particular interest to us at this stage is the great difference in the solubility of both substances in water. The preparations used dissolved in water (Aqua dest. 18° C) in the following ratio:



The commercial preparation, the two pure "Systox" isomers and the emulsifier were all tested in these studies. Furthermore, several tests were conducted with triethyl phosphate which occurs as a secondary constituent. Octamethyltetrapyrophosphoramidate ("Schradan") was also included in these studies by way of comparison. The portion of active substance in the preparation used amounted to 66 %.

2. Culturing of algae

In order to fully exploit the advantages offered by the laboratory experiment, simply organized test organisms were used, in particular unicellular green algae. These unicellular organisms may be cultured at any time under conditions which can be exactly defined. Moreover, they have the advantage over higher plants that their gas exchange is not obliterated or distorted by the influence of stomata, intercellular spaces and other factors. However, experience gained with such standard objects cannot be applied without criticism to practical conditions prevailing among cultivated plants.

The test organism was a strain of *Ankistrodesmus braunii* (Nägeli) Brunnthaler isolated in the Marburg Botanical Institute, which had already been used quite often in physiological studies (Pirson, Tichy and Wilhelmi; Pirson and Kellner; Kessler; Krollpfeiffer). In addition to *Ankistrodesmus*, a strain of the green alga *Chlorella vulgaris* (Beijerinck) was also included in several tests (Pringsheim collection No. 211/11 h). The algae were cultured under sterile conditions on Knop agar slants, and transferred from these stock cultures to the culture solution specified by Pirson et al. in accordance with Alberts-Dietert.

Unless otherwise mentioned, the experiments were conducted in a nutrient solution containing nitrate as the source of nitrogen. The nitrate solution offered two methodical advantages for these studies:

1. This nutrient solution is buffered better in the pH range between 4 and 5 than the corresponding ammonium solution. Consequently when the pH is adjusted by means of diluted phosphoric acid, fewer difficulties are experienced with NO_3 solution than with ammonium solution. In this range, it is easier to displace the pH of the latter.
2. It proved necessary to transfer the algae to fresh solution at the start of each experiment. No difficulties are encountered with a culture in nitrate solution whereas ammonium cultures mostly flocculate following such treatment. This disturbance can be precluded by stirring the centrifugate of algae and allowing it to drop from the centrifuge tubes into the test solution.

500 ml flasks made of Jenaer apparatus glass (G 20) were used for the nutrient solution cultures. Culturing was carried out in continuous light (incandescent bulb, light intensily in the range of the flasks, 800 lux), and at a temperature of approximately 24° C in the culture flasks.

The cultures were aerated with a mixture of air and CO_2 (6 % CO_2). The air and carbon dioxide were taken from steel cylinders, and then passed for cleaning and water-saturation through several cotton-wool filters and a number of washing flasks (with sintered glass filters) containing water or, alternatively, potassium permanganate solution. In order to be able to keep a constant and exact check on the mixing proportion of the gas, the current of air and CO_2 was passed through a capillary flowmeter. Approximately 5 litres of the mixture of air and CO_2 flowed through the individual culture flasks every hour.

5 to 7 day old cultures were used for the experiments. The quantity of inoculum amounted to 0.5 mg dry weight in each case. For the experiments, the algae were centrifuged from the culture medium, and transferred to fresh nutrient solution. Whenever necessary ($\pm$ KOH method, photosynthesis measurements), the test solutions were adjusted with diluted phosphoric acid to pH 4.0–4.5. By tipping in trichloroacetic acid, it was occasionally established at the end of the experiment that the pH had still not increased to the retention range.

Parallel measurements were carried out on leaves of the Canadian water-weed (*Helodea canadensis* Rich.) which may be cultured without difficulty in the usual manner in the aquarium with tap water. The culturing of the *Tradescantia* cuttings which were used does not involve any special work, either. Sugar beet roots were used only for a few comparative measurements because the task of culturing would have been too laborious here. According to the method described in the literature, the monogerm seed is placed on slant agar for germinating (cf. v. Rümker). 10 to 15 mg dry weight substance were required per test flask, in other words approximately 150 mg per test (12 flasks).

3. Manometric method

The use of the manometric method appeared advisable because the results already available from past experiments did not give rise to the expectation that there would be any very strong physiological effects after doses of "Systox" are applied and, therefore, a method had to be selected which would permit even the smallest of variations to be recorded with accuracy within a minimum of time.

The manometric experiments were all conducted at 24°C and, unless otherwise stated, with 8 ml of algal suspension in rectangular vessels (22 ml). The Warburg apparatus Type S fitted with a lighting system and manufactured by Messrs. Braun, Melsungen, was used for the quotient determinations, whereas the other measurements were taken on an apparatus of a more simple design (vibration frequency approx. 120/min., amplitude 3 cm).

The respiratory consumption of O_2 was determined according to the direct KOH method as the standard for the respiration intensity. The $\pm$ KOH method was employed to determine the RQ ($-\text{CO}_2/\text{O}_2$), and the vessel difference method (cf. Pirson, Krollpfeiffer and Schäfer) for determining the PQ ($-\text{O}_2/\text{CO}_2$). All the other photosynthetic measurements were carried out according to the direct two gas method (assuming $\gamma = -1$). The formulae used are given in the appendix. The derivation of the formulae and a detailed discussion of the method may be found in the papers of Krebs, Umbreit, Burris and Stauffer, and Dixon.

In the photosynthetic measurements (with the exception of the quotient determinations), four fluorescent lamps were placed at a distance of approx-

imately 45 cm above the flasks to illuminate the experiment. A tank coated with white radiator enamel was used as the reflector. Two mirrors placed underneath the vessels improved the light efficiency. The inside surfaces of the water bath were coated white.

The fluorescent lamps used were of the Philips HPL type (125 watt). The spectral energy distribution of these lamps is illustrated in Fig. 1. Fluorescent lamps of this type are a great advantage in that the experiment can be well illuminated without the water bath being heated to any considerable extent, an otherwise disturbing factor. The reason for this is that the lamps do not radiate any energy worth speaking of above a wave range of approximately 700 m μ . With the experimental apparatus arranged in the manner selected, 8,500 to 9,000 lux was measured under water in the range of the vessels. It was shown that the light saturation range required for the algae was actually produced under the given conditions.

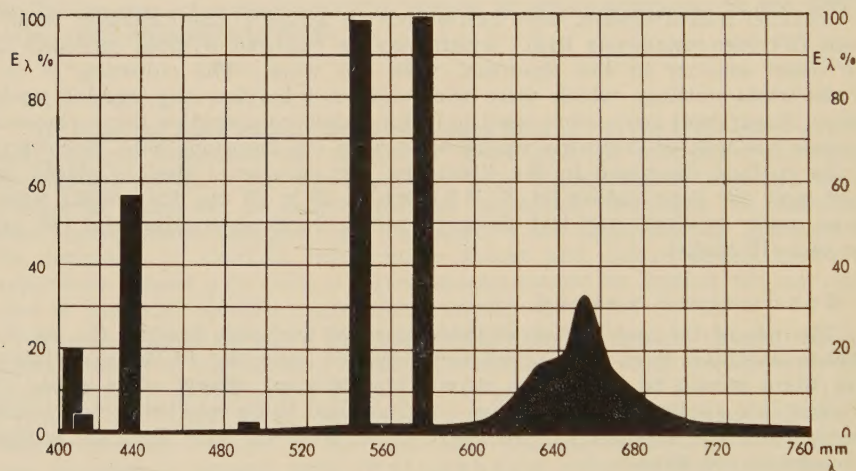


Fig. 1: Spectral energy distribution of Philips HPL lamp, 125 watt (according to data of Philips A.G., Hamburg).

4. Tracer method

A ^{32}P -labelled "Systox" ($\text{P} = \text{S}$) compound was used in a number of tests. A detailed description of the tracer method would be superfluous within the scope of this paper. Full methodical data are given in the publications of Schubert et al., Martin et al., and Russel and Martin. Special methodical hints for working with labelled "Systox" are given by Tietz.

The measuring apparatus used was developed in the Physics Laboratory of Farbenfabriken Bayer Aktiengesellschaft (Leverkusen) (Dr. Sybertz). The counting apparatus had a 32 fold reduction gear. The weak radiation of phosphorus renders it necessary to establish a close contact between the substance being measured and the countertube. Therefore, a liquid countertube manufactured by Messrs. Friesecke and Höpfner (Erlangen) was used. The countertubes employed had an efficiency of approximately 8 % for ^{32}P .

The measurements were taken at a countertube voltage of 1,280 volts. With a voltage range of the countertube amounting to 1,020 to 1,400 volts, the guarantee was given that the measured values were independent of any changes in voltage. Each measured value represents the average of three measurements lasting 5 minutes. Each individual measurement was preceded by a zero effect measurement lasting 3 minutes. All measurements carried

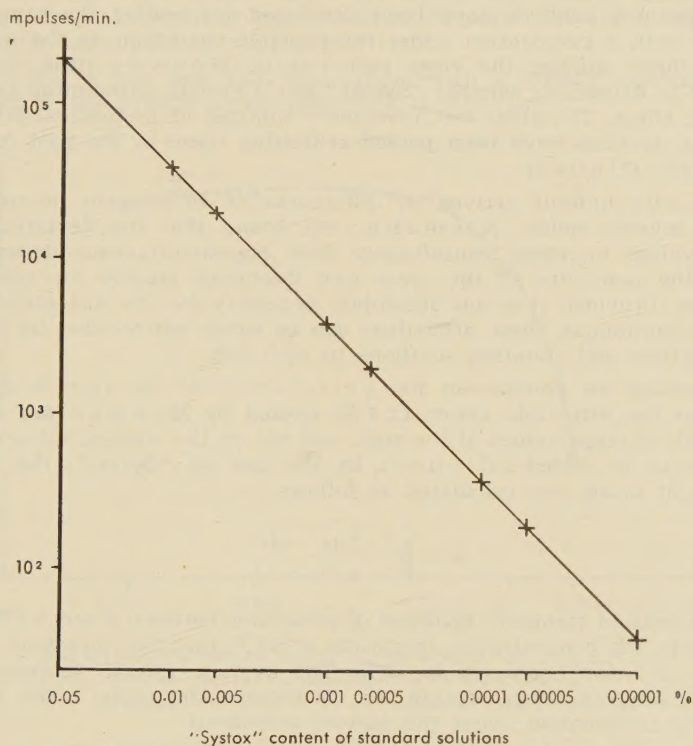


Fig. 2: Measured impulses at different concentrations of ³²P-labelled "Systox" standard solution.

out were relative. Consequently it was not necessary to calibrate the counter-tube and to determine the specific activity of the preparation. Finally, the corrections for absorption and self-absorption necessary with absolute measurements, are precluded when an equal quantity of measuring liquid (10 ml) is constantly used.

In order to be able to compare the measured values, the values read should be corrected three times (Schubert, 1951) (cf. appendix for the formulae used for correction). These three corrections were made to all the measured values obtained. The impulse figures obtained after the corrections have been made, should be expressed as a function of the preparation content. This function may be illustrated for the preparation used in the manner depicted in Fig. 2. The values given in this graph were obtained from the

measurements of standard concentrations. The test solutions were measured in the same way as the standard concentrations. The ^{32}P content of the test solution can be determined direct by comparing the resultant impulse figures with the results of the standard measurement according to Fig. 2.

5. Cress root test

Several test methods have been developed for testing the compatibility of plants with a preparation under reproducible conditions in the laboratory test. In these studies, the cress root test of Moewus (1948, 1949) was employed to determine whether "Systox" has a growth stimulating or growth inhibiting effect. The cress test involves a number of methodical difficulties, and many opinions have been passed criticizing these in the past few years (Reinert, Clauss).

Moewus himself arrived at differences of 20 % when he used cress seeds of several yields. Niemann, too, found that the deviation of the average values increased considerably with non-simultaneous determination because the reactivity of the cress root fluctuates greatly for reasons yet unclarified. Provided it is not absolutely necessary for the test series carried out to be continuous, these difficulties can be easily surmounted by including water controls and standard solutions in each test.

In passing an opinion on the efficiency of the test, it should be noted that the three-fold errors ($\pm 4\%$) quoted by Moewus are based on the growth increase values of the root, and not on the concentration of active substance to be tested (Clauss). In the test on "Systox", the standard deviation of mean was calculated as follows:

$$m = \sqrt{\frac{\sum (x - M)^2}{n \cdot (n - 1)}}$$

The threefold standard deviation of mean was between 4 and 5.5 %. It was merely with the concentration inhibiting at 40 % that the threefold standard deviation of mean increased to 10 %. The average growth increase in the controls lasting 18 hours was 16 mm. Reference should be made to Niemann for information about the general method.

Cress seeds of Messrs. Mildebrandt (Cologne), 1953 harvest, were used. The tests were not conducted in ordinary Petri dishes but in Petri dishes with ground lids. In this way the test solution was prevented from evaporating too quickly in the dishes. Moreover, the disturbing effect of gaseous substances is precluded to a greater extent than when ordinary Petri dishes are used. Niemann emphasizes the importance of this in his paper. In my own tests, the standard deviations of mean were always higher with ordinary Petri dishes than in the test series where dishes with ground lids were used. All the glass equipment used was rinsed thoroughly with a detergent and not with chromosulphuric acid.

The thermostat which was used indicated fluctuations of $< \pm 1^\circ$ every 20 minutes. The commercial preparation was tested at all concentrations. It was established in preliminary tests that the pure emulsifier has neither a growth stimulating nor a growth inhibiting effect in the concentration ranges tested. Each measuring point is the average of 40 single measurements (= 4 test vessels).

III. Experimental section

A. Growth measurements

1. Testing of the systemic insecticide "Systox" in the cress root test

The cress test was used to establish first of all whether in certain concentration ranges the cell growth processes, in particular cell elongation, are inhibited or stimulated by "Systox".

From Fig. 3 it is seen that in solutions with a "Systox" content of more than 0.005 %, the growth of the cress root is inhibited.

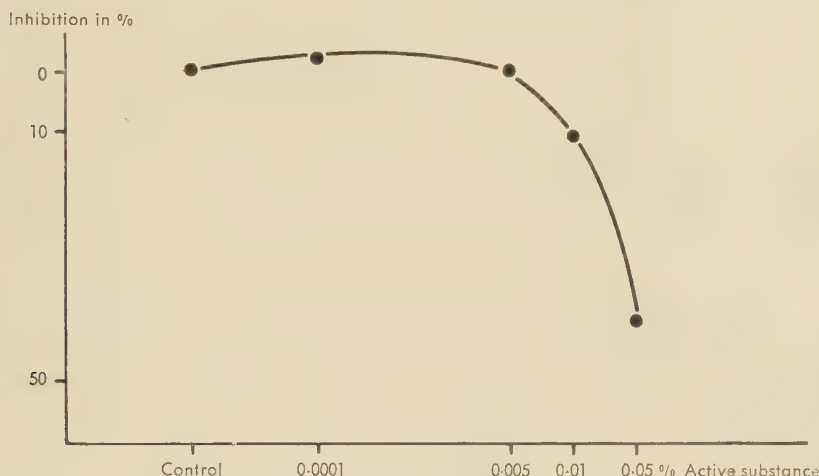


Fig. 3: Testing of different "Systox" concentrations in the cress root test.

Abseissa: Active substance content of the tested solution.

Ordinate: Inhibition of the root growth of cress expressed in %
(water controls = 0%).

Standard deviat. Control	0.0001 %	0.005 %	0.01 %	0.05 %
1.3 %	1.8 %	1.85 %	1.6 %	3.4 %

The curve has a certain resemblance to the corresponding curve for "Folidol E 605" conc. (Beck). With "Systox", the inhibition first starts one complete power of ten later than with "Folidol E 605". The curve is unusually steep between 0.005 and 0.05 %, i. e. only a slight dilution is required in this range to considerably reduce the inhibiting effect. A stimulation of growth does not exist in the range of low concentrations.

Further studies are necessary to establish to what extent an occurrence of inhibiting concentrations must be expected when the preparation is applied by the soil watering technique (cf. P. 299).

When the curve published by Beck for "Folidol E 605" is compared with the limit concentrations published by Frohberger for plant injuries caused by this preparation, it is evident that the cress root apparently reacts to those concentrations which are well tolerated by the fully grown plant

without any noticeable harm being inflicted. A similar displacement of the reaction limit value is also to be expected with "Systox" according to practical experience (Unterstenhöfer, 1952; Bosse).

2. Effect of stronger "Systox" concentrations on the growth of the *Lemna* root

With the objects previously used in growth regulator tests, including the cress root, it is very difficult to say whether an observed effect is actually due to an inhibition or stimulation of the cell elongation. Cell division and elongation are not easy to separate in this respect.

According to results published to date, far more favourable conditions are offered by the root of the duckweed (*Lemna minor*). During the past few years, *Lemna* has often been used for metabolism and cell-physiological studies (Pirson and Seidel, Pirson and Göllner, Göllner).

According to the method of Göllner, the total growth increase of the root is measured on the floating plant with the aid of a horizontal microscope. Immediately afterwards, the number of new cells formed in a certain location during the test period are counted on the same object, and the average cell length is determined. Cell division processes and cell elongation are thus recorded separately.

In the spring of 1954, Miss Reininghaus of the Botanical Institute, Marburg, employed this method to test a number of substances for their effect on the *Lemna* root. These tests, which previously had not been published, also included the "Systox" P = S isomer. The results are given in Table 1. As it was intended to apply the preparation as an emulsion, the pure emulsifier was also tested here for its effectiveness.

Table 1: Effect of different "Systox" concentrations on the root of *Lemna minor*.
Test period 48 hours, 22° C, 700—800 lux.

Concentration %	Growth increase/h	Cells formed/h	Average cell length in μ
0.005	Serious damage to the cells		
0.001	15.9	186.6	85.2
0.0005	17.6	215.5	81.7
0.0001	21.7	252.5	85.9
Control	21.2	246.0	86.2
Emulsifier 0.01 %	21.8		

Just as in the cress test, the total growth increase per hour also drops here from a certain limit concentration onwards. The other observations show that the cell division processes are influenced more than the cell elongation processes. A comparison with the cress test reveals that the *Lemna* roots react to concentrations at which the cress roots displayed no reaction whatsoever.

Such tests for determining an inhibiting or stimulating effect on the cell growth processes are often referred to as "plant compatibility tests" (Allen

and Casida). It is, however, most certainly unjustified to say that a preparation is well tolerated by plants merely because it behaves indifferently in the growth regulator test. When judging the plant compatibility of a preparation, the influence exerted upon a single isolated growth process or metabolic process, is often of less interest than a possible displacement of the total balance of metabolism under the effect of the preparation. Therefore, if an opinion is to be given on the question of the plant compatibility of a preparation, then it is absolutely essential to make an exact check on the influence exerted upon different and, wherever possible, central metabolic processes.

3. Increase in the dry weight of suspensions of unicellular algae

It was proposed to use the green alga, *Ankistrodesmus braunii*, as one of the test organisms in subsequent studies, a fact already referred to above. Therefore, it was essential to establish first of all whether, and in what concentrations, this alga species tolerates the preparation without suffering any harm. It was to be expected that the unicellular organisms which come into direct contact with the preparation during the tests through their relatively very large and unprotected surface, would react more sensitively than a higher sprayed plant.

A really good general survey of the overall physiological influence of a factor is obtained when a study is made of its effect on the increase or, alternatively, the decrease of the dry weight production. Determinations of the increase in dry weight can naturally be carried out far more exactly with algal suspensions than with higher plants. On the one hand, in the examination of algal suspensions the experiments can always be started again with a well defined, equal initial quantity of plant material and no difficulties are involved, while on the other hand there are no individual differences whatsoever between each test object. In this case, the "test object" comprises the entire culture flask with the large number of single cells which statistically have a uniform reaction. Provided the flasks are equally supplied with light, nutrient salts and CO₂ and that the test temperature is constant, it should be possible to attribute all differences occurring among the individual cultures to the variation factor in the test.

Methodical hints on the determination of dry weight for algae are given in the publication of Pirson et al. (1952). At the beginning of the experiment, each culture flask (180 ml) was given a quantity of inoculum which corresponded exactly to a dry weight of 2 mg. In these cultures, the dry weight was then determined for 25 ml of the test suspension during the course of the experiment.

The algae developed so rapidly at an average temperature of 24°C in the flasks that it was possible to obtain reliable values in the dry weight determination as early as the second day following the inoculation. Fig. 4 gives a summarized illustration of two experiments. The dry weight production was examined in the presence of different concentrations of a "Schradan" preparation and in the presence of "Systox".

The experiment shows that at low concentrations, the increase of dry weight in the controls largely corresponds to the increased values in the preparation solution. It is not until a "Systox" concentration (active substance)

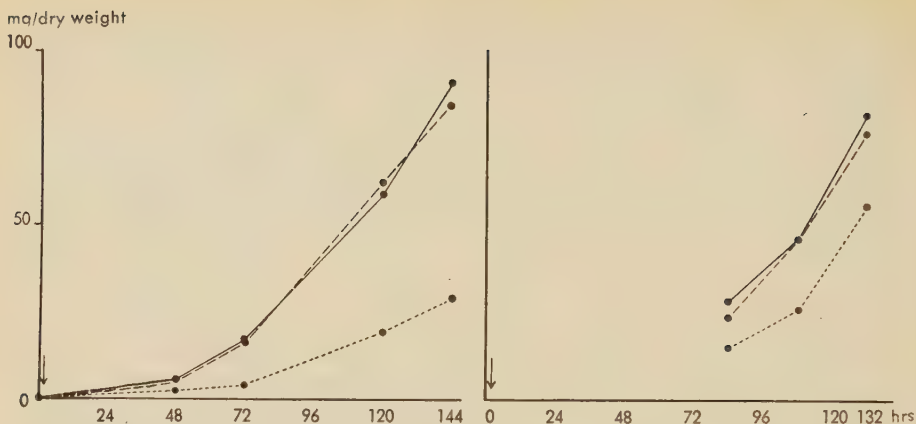


Fig. 4: Dry weight production for *Ankistrodesmus* in "Schradan" (left) and "Systox" solutions (right), expressed in mg dry weight/ml.

— Control
 - - - 0.001 % "Schradan" or 0.0005 % "Systox" (active substance)
 0.014 % "Schradan" or 0.005 % "Systox" (active substance)

Abscissa: Age of culture in hours. Inoculation of 2.0 mg dry weight/180 ml nutrient solution in the first hour.

is used which corresponds to the inhibiting concentration in the cress root test that the dry weight production is also clearly reduced. Both for *Ankistrodesmus* and for the cress root, the limit of the harmful concentration is, therefore, almost one whole power of ten higher than for the susceptible *Lemna* root (cf. P. 274). The "Schradan" preparation must be slightly stronger than "Systox" before it inhibits the dry weight increase. Here, it should be taken into consideration when passing a practical opinion that "Schradan" preparations have to be applied in higher dosages because they first become fully effective insecticidally at higher concentrations than "Systox". Therefore, it is advisable when testing both preparations, not to compare the same concentration but to compare concentrations of equal insecticidal potency. The manufacturers recommend the application of "Systox" at a concentration of 0.025 % (active substance) against spider mites, whereas the makers of the "Schradan" preparation which was used in these studies recommend for their product a concentration of 0.05 % (active substance) for controlling the same pest. The tested "Schradan" concentration of 0.014 % active substance content, therefore, corresponds to a "Systox" concentration of 0.007 % as regards its biological efficacy, so that it must be compared with a similar concentration here, too.

As mentioned above, the commercial preparations, and not the pure active substances, were tested in these experiments. Here too, it had to be determined first of all in preliminary tests to what extent the emulsifier had influenced the test result. Several experiments showed that cultures to which the emulsifier was added at concentrations ranging from 0.0001 to 0.007 %, do not differ from the controls in their behaviour. On the other hand, the active substance without an addition of emulsifier did not act any differently from the way it acted when the appropriate portion of emulsifier was added.

Table 2: Dry weight production of *Ankistrodesmus* in a "Systox" solution, an emulsifier solution, and a control solution without preparation (mg dry weight / 100 ml).

Time interval following inoculation of culture flasks	"Systox" 0.0005 %	"Systox" 0.007 %	Emulsifier 0.007 %	Control
84 hrs.	24	15	31	29
132 hrs.	72	56	78	82

4. Morphological changes of *Ankistrodesmus* cells in "Systox" solutions

The algal suspension was aerated with a mixture of air and CO₂ both in the cultivation of the algae in the preparation solution as well as in the otherwise normal cultivation in a standard solution. A fairly large amount of preparation was blown out by the air current during the course of several days. By means of a radioactive labelled preparation, it was established that after 6 days approximately half of the active substance had been forced out of a 0.0005 % solution, while about two-thirds of the preparation were forced out of a 0.005 % solution in the same period.

The preparation content in the solution, therefore, decreases considerably as the result of normal aerating of the culture flasks. In fact this decrease is more rapid with a high initial concentration than with a low strength.

In order to make it possible for high "Systox" concentrations to act on the cells also over a long period, the air-CO₂ mixture used for aerating the culture flasks was passed through 2 washing flasks filled with a saturated "Systox" solution (without emulsifier; several drops of preparation in excess). Consequently the preparation was no longer expected to be blown out of the solution.

It was no longer possible to determine an increase in dry weight in the cultures treated in this manner. However, when the algae were transferred back to a fresh solution free of "Systox" after 6 days had elapsed, the growth process very soon returned to normal.

There are several influences which lead to disturbances of the cell division processes in *Ankistrodesmus*. When potassium is replaced in the nutrient solution by rubidium, greatly enlarged and abnormally shaped cells are observed in the cultures (Pirson and Kellner). Similar disturbances were also observed in the cultivation of algae in glucose and sucrose solutions (Chodat, Vischer). Kessler (2) also observed such giant forms when working with *Ankistrodesmus* in acid solutions. Occasionally abnormal cells, e. g. three-pointed, occur with over-aged cultures. It, therefore, appeared advisable also to make a microscopic examination of the cells apparently damaged in the highly concentrated "Systox" solution, for their morphological changes.

It was found after only 48 hours of cultivation in a 0.02 % solution, that the cells appeared very much larger in comparison with the controls, whereas the cell division process had practically stopped (size measurement by means



Fig. 5: *Ankistrodesmus* in standard nutrient solution; division stages.

Notes on the photographs:

Lens: Leitz, $\frac{1}{12}$ Oil immersion, 100:1, A: 1.30

Ocular: Leitz, 10 $\times$, Periplan

Leica: Micro attachment $\frac{1}{3}$ $\times$

Filter: BG 34 (Schott and Gen.) Re-enlargement to 700 $\times$

of the drawing prism, counts made in the counting chamber according to Thoma). After a culture period of 120 hours, the cells in the preparation solutions had developed to several times their normal volume. In contrast to the giant cells illustrated by Pirson and Kellner, the forms observed here never had a blistery, deliquescent outline. Occasional cross-segmentation of the plasma could be interpreted as disturbed division (Figs. 6 and 7).

It has already been mentioned above that the badly damaged cultures soon begin to grow normally again after they are transferred to fresh nutrient solution. When these "reconvalescent" cultures are examined under the microscope, typical giant forms are observed which later disintegrate into a large number of small cells in a "Systox"-free solution. In their volume, these small cells are more or less normal (Fig. 8). Subsequent divisions of these normal sized cells again produce the picture of cell division characteristic of *Ankistrodesmus* (Fig. 5). Cells of this origin were cultivated for a further period of several weeks in a nutrient solution, but there were no signs of abnormal behaviour.

Apparently the damage discussed here causes a stoppage of the normal cell division processes accompanied by inhibited plasm growth, and leads to the formation of the giant cells illustrated here. However, such injuries are not so



Figs. 6 and 7: *Ankistrodesmus*, cells cultivated 120 hours in 0.02% "Systox" solution. Phototechnical data as for Fig. 5.

serious as to cause any adverse effects on the normal behaviour of the cells of the following generation.

The occurrence of giant cells in *Ankistrodesmus* must admittedly be regarded as a non-specific and general reaction although reference should be made here to the coincidence of these results with the findings made on the *Lemna* root. In both cases, it was not so much the plasm growth as the cell division that was disturbed at first.

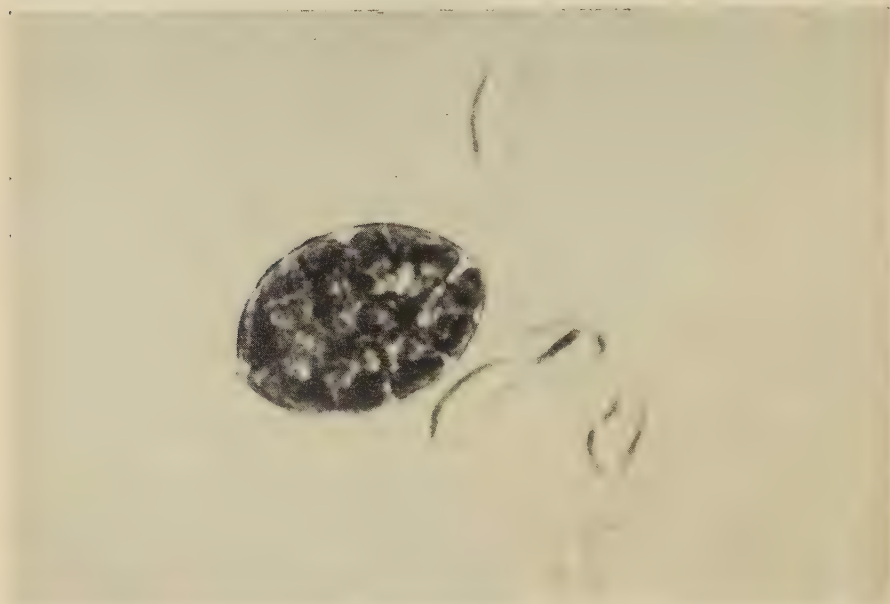


Fig. 8: *Ankistrodesmus*, cell division stage, cells from a "Systox" culture (0.02%), 3 days after transfer to a "Systox"-free nutrient solution. Phototechnical data as for Fig. 5.

B. "Systox" concentrations in algae poor in fat and in those which store fat

In all previous tests, the concentration of the applied solutions was always regarded as a standard for the "Systox" concentrations which become physiologically effective. In order to gain an approximate idea of the quantities of "Systox" actually present in the cells in these tests, the content of "Systox" (or of ^{32}P) in the algae was determined in several cases by using a ^{32}P -labelled preparation.

In order to make a reliable measurement of the activity of a plant tissue, it is essential to place the material being examined in the solution by means of a method which will ensure that there is no loss of measurable quantities of the labelled atom. For this purpose, use is made of the so-called liquid ashing with concentrated sulphuric acid and perhydrol (cf. Tietz).

Approximately 6 ml of conc. H_2SO_4 are poured over an exactly measured quantity of the centrifuged cells in a fire-proof centrifuge tube. The tube should then be heated

on a sand bath until the algae dissolve completely, and a uniform brownish-black liquid is formed. The carbon left over after the sulphuric acid has acted, is then oxidized by adding 2 to 3 ml of perhydrol. The solution then becomes as clear as water. Finally the solution should be filled up to 10 ml with Aqua dest.

Each of the samples produced in this manner contained approximately 50 mg of algal dry weight substance. After these samples had been measured, the absolute content of "Systox" in the cells per unit of dry weight was determined by comparing the values with standard measurements.

a) Tests with normal cells

Ankistrodesmus cells were transferred for three hours to a 0.01 % solution of "Systox". The cells were then washed out three times with distilled water to remove any residues of the preparation adhering either between the centrifuged algae or to their surfaces. Immediately after they had been washed, a sample of the cells (Table 3 A) was subjected to wet combustion, and the content of ^{32}P was determined.

Another sample (B) was transferred to a "Systox"-free nutrient solution after it had been washed, and thoroughly shaken in it for five hours. Then the content of labelled phosphorus present in these cells was determined. In addition to the combusted solution, the original preparation-free nutrient solution was also measured, from which the algae of sample B had been centrifuged. This was done to confirm the results obtained with sample B. Table 3 gives a summary of these tests.

Table 3. "Systox" content in *Ankistrodesmus* cells after being kept for 6 hours in a 0.01 % emulsion of the preparation.

A Algae (100 mg dry weight) washed out three times in "Systox"-free solution	B Algae (100 mg dry weight) washed out three times in "Systox"-free so- lution, and then thoroughly shaken for 5 hours in "Systox"-free solution (20 ml)	C Solution centrifuged after algal material had been shaken for 5 hrs. in test B
0.453	0.224	0.136
0.450	0.272	

Numerical data: mg "Systox"/100 mg dry weight

mg "Systox"/20 ml solution

When the algae are centrifuged and washed three times, there is no doubt that a large percentage of "Systox" disappears from the cells. Therefore, the quantities of "Systox" actually present in the cells are in all probability greater than the values given in Table 3 A. However, it will be appreciated that a thorough washing must be carried out.

A comparison of groups A and B reveals that within 5 hours approximately $\frac{1}{3}$ of the preparation stored in the cells was liberated into the "Systox"-free test solution. This percentage is mostly found again in the test solution (column C). The slight deficit is due not only to measuring errors but also to the fact that a small percentage of the preparation was evaporated from the solution into the gas volume of the reaction vessel or, alternatively, it adhered to the glass of the vessel. The findings given here will be discussed later in explanation of several test results (cf. P. 293).

b) Tests with fat-storing nitrogen-deficient cells

When *Ankistrodesmus* is cultivated in a nutrient solution containing only $\frac{1}{5}$ of the quantity of nitrogen normally added (the deficient KNO_3 is compensated for osmotically by KCl), the chlorophyll content soon decreases after a good initial growth of the culture. After several weeks, the microscopical examination will show that the cells, which are somewhat larger than normal cells (Fig. 9), are completely filled with small droplets (Fig. 10). These droplets are easy to identify as fat droplets by staining them with "Sudan III" (§ 1045). The algae are extremely resistant in this state, and their metabolism decreases



Fig. 9: *Ankistrodesmus*, normal cells.

considerably (Brüggemann). They are coloured orange-yellow by the secondary occurrence of carotenoids. Such cells, however, are not seriously damaged as is shown by the fact that they become green again within two days following the addition of different sources of nitrogen (NH_4Cl , NaNO_3 , NaNO_2) (Kessler).

It is, therefore, possible to examine, in comparison with the tests described on Page 281, the penetration of "Systox" into the cells of *Ankistrodesmus* and the washing out process of the preparation from these cells in algae which store large quantities of fat and which incidentally correspond to the organisms used in the experiment described on Page 281.



Fig. 10: Cells of *Ankistrodesmus* deficient in nitrogen. — Phototechnical data as for Fig. 5.

Cells deficient in nitrogen which were heavily loaded with fat droplets (Fig. 10, deficient in nitrogen for 3 months), were pre-treated in exactly the same manner as the cells used in the experiment summarized in Table 3. Sample A was subjected to wet combustion after having been centrifuged and washed three times (cf. P. 281). Sample B, on the other hand, was washed three times and then transferred to a "Systox"-free nutrient solution where it was thoroughly shaken for 5 hours, just as in the experiment of Table 3. Afterwards this sample, too, was subjected to wet combustion in the usual manner. The results of the two parallel experiments are given in Table 4.

Table 4: "Systox" content of *Ankistrodesmus* normal cells and of fat-storing algae deficient in nitrogen after being left in a 0.01 % "Systox" emulsion for 3 hours.

A		B	
Algae of 100 mg dry weight, washed three times in "Systox"-free solution		Algae of 100 mg dry weight, washed three times in "Systox"-free solution, then shaken five hours in "Systox"-free solution	
Normal cells	Cells deficient in nitrogen	Normal cells	Cells deficient in nitrogen
0.453	0.700	0.294	0.634
0.450	0.770	0.272	0.604

Numerical data: mg "Systox"/100 mg dry weight

Apparently more preparation is absorbed by the nitrogen-deficient cells rich in fat than by the normal cells. The fat-storing cells contain over 50 % more ^{32}P than the fat-free algae, and the difference increases considerably with continued washing. After being left for 5 hours in a "Systox"-free nutrient solution, the ^{32}P content in the normal cells, in contrast to the N-deficient cells, decreases so much that the cells rich in fat now contain 100 % more labelled substance than the normal algae. Consequently it is far more difficult to wash the preparation out of the fat cells than out of the normal cells.

Despite these striking differences, it is surprising that they are not even greater when it is considered that "Systox" is extremely lipophile and unpolar (Schradler). One would almost expect that a separation by solvent extraction of the preparation from the culture medium would occur with these fat-storing algae. The fact that only a limited percentage of the preparation is absorbed by the algae, might be regarded as an indication that the surface layers of living cells play a certain part in the incorporation of the preparation, and that the passage of the "Systox" molecule into the cell is not merely a flushing in of the ester. There are also several other results on record which serve to oppose the argument that the cells behave purely passively when the systemically active preparations penetrate into the cells. It was observed that both "Systox" (Tietz) and "Schradan" (David) can under certain circumstances be excreted by the root in the same solution from which the preparation was first absorbed. In explanation of these findings, it may be assumed that there is a change in the adsorption and permeability conditions in the cells or, alternatively, in the membranes due to the effect of the preparation, or else that there are certain rearrangements in the active substance molecule which result in a change taking place in the solution or adsorption properties of the preparation.

C. Measurements of respiration

The results so far published of experiments conducted on algae in solutions of different "Systox" concentrations show that the preparation at concentrations over 0.005 % active substance influences the balance of metabolism of these organisms in some form or other. In order to analyze these effects more closely, the respiratory O_2 absorption of *Ankistrodesmus braunii* was determined in a normal nutrient solution with "Systox" present in different concentrations.

1. Respiration in "Systox" concentrations of 0.0001 to 0.01 %

To begin with, solutions containing very little preparation were tested, using concentrations of 0.0001 to 0.004 % active substance. The commercial preparation *, in other words 50 % emulsifier, was used in these tests. Here too, just as in previous experiments, it was established that the pure emulsifier does not cause any disturbing effects.

* Use was always made of the same production batch in all other tests with the "commercial preparation".

Table 5: Respiratory O₂ consumption by *Ankistrodesmus* in solutions with a "Systox" content of 0.0001 to 0.004 % active substance. Direct KOH method.

Preparation content of solution	0.0001	0.0005	0.001	0.004
Respiration over	2.4	3.1	2.4	2.8 + preparation
3 hrs. — mm ³ O ₂ /mg/h	2.5	3.2	2.4	2.5 control

The tests were not all carried out at the same time, and it is for this reason that a special control value has been given for each concentration. The greater respiration of the algae in the second experiment may be explained by the fact that here a two day younger suspension was used.

At a "Systox" content of 0.004 %, the respiration increased in comparison with the control in four tests. This increase, however, was always very slight. In all the other cases, at concentrations of below 0.004 % active substance, there was neither an increase nor a depression in the respiratory O₂ consumption in comparison with the control.

In view of the slight increase of respiration in a 0.004 % "Systox" solution, it appeared advisable to test higher concentrations. As already mentioned earlier, the limit value for a weak disturbance was found to be in this range both in the cress root test and in the dry weight determinations.

The greatly varying solubility in water and also certain differences in the animal physiological efficacy displayed by the two isomers present in "Systox" indicated that it would be advisable to test the two isomers separately in future phytophysiological experiments.

Fig. 11 shows the respiratory consumption of oxygen by *Ankistrodesmus* in a 0.01 % "Systox" solution as the standard for the intensity of respiration. The efficacy of the pure P = S compound and the pure P = O compound was examined separately. Immediately the P = S compound had been added, the respiration of the algae increased to a value almost 100 % greater than the comparable value of the control. The rapid increase is particularly amazing since the active substance which is not very soluble in water is a relatively large and bulky molecule. Apparently the preparation is capable of penetrating very quickly to the site where it becomes effective in the cell. It is quite probable that the mechanism of action is localized in the lipophile structures of the surface layers of the cells.

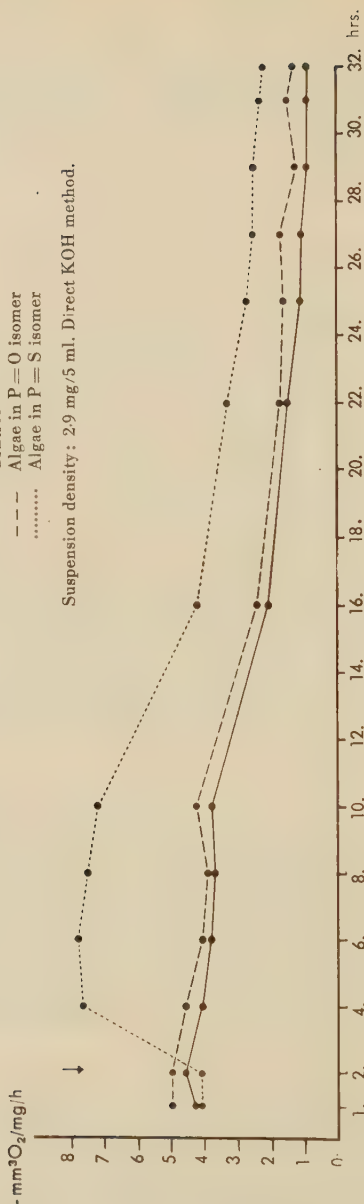
Moreover, it is most surprising that in contrast to the P = S compound, the P = O compound which chemically is so very closely related, did not influence the O₂ consumption in any way whatsoever. After about 25 hours the relative difference between the O₂ consumption in the control and in the P = O compound had increased, whereas the difference between the P = S isomer and the control remained more or less the same. However, as the respiration decreased here to a really low final value following a gradual consumption of the respiratory substrate, the measured values were affected in their degree of exactitude. It is, therefore, difficult to guarantee the differences with reliability.

Fig. 11:

Ankistrodesmus, respiratory O_2 consumption after addition of 0.01% "Systox" (+ emulsifier). Addition after 2 hours had elapsed (arrow).

— Control
 --- Algae in P=O isomer
 Algae in P=S isomer

Suspension density: 2.9 mg/5 ml. Direct KOH method.



Studies made by Gaffron and Kessler (2) on different unicellular organisms showed how differently related forms react at times. Experience gained with one object cannot, therefore, be applied generally, in fact not even within small systematic units.

In order to place the results obtained here on a somewhat broader basis, the behaviour of *Chlorella* "11 h" was examined in comparison with that of *Ankistrodesmus*. These tests produced results which basically were similar to those obtained in the experiment illustrated in Fig. 11. The reaction of *Chlorella* to the influence of the preparation will be dealt with in greater detail later (Fig. 18). In order not to restrict the studies solely to unicellular organisms and algae, *Helodea canadensis* was included in the experiments as a simply organized monocotyledon. *Helodea* is particularly suitable in this case for methodical reasons.*

Fig. 12 shows that following the application of the preparation, the respiratory consumption of O_2 by *Helodea* increases to the same extent as with the other objects. *Helodea*, however, appears to be somewhat more sensitive than the unicellular organisms which were examined. Whereas there was an increase of about 40 to 70 % in all cases with algae even at a concentration of

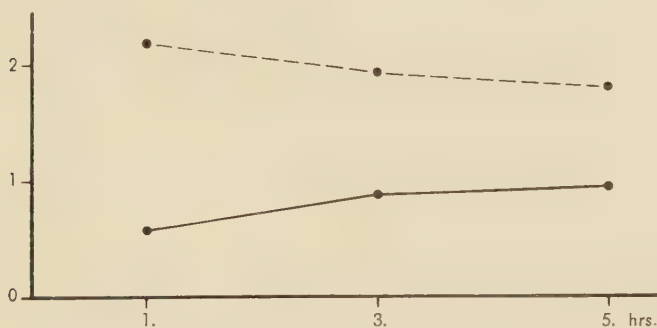


Fig. 12: *Helodea canadensis*, respiratory O_2 consumption in 0.02% "Systox" solution (+ emulsifier), P = S isomer. Transferred to the test solution one hour before the measurement was started.

— Control
 --- Leaves in "Systox" solution. Direct KOH method.

only 0.007 %, *Helodea* did not react clearly at this strength. This may be partly explained by the fact that the preparation settles most obstinately on surfaces, so that consequently it accumulates much more rapidly and in greater quantities on the unicellular organisms with their relatively large surfaces. The pure P = O compound did not have any effect on the extent of the respiratory O_2 consumption of *Helodea*, either.

2. Effect of several substances related to "Systox"

The large difference in the effect of the two isomeric substances noticeable in all previous tests may be due to the very great difference between both preparations in their solubility in water. Allowance should also be made for a specific effectiveness of the phosphorus-sulphur double bond together

* Gassner also used *Helodia* to examine the phytophysiological effect of several systemically active urethane derivatives.

with the other molecules. The conditions here would then be just the opposite to those in the animal test where the $P = O$ isomer of "Systox", "Folidol E 605" and other esters of this group always proved to be considerably more toxic than the corresponding $P = S$ compound (Hecht and Wirth).

If it were to be established that the two "Systox" isomers actually do differ so greatly in their effectiveness on the respiratory gaseous metabolism in the plant, as would appear to be the case from past experiments, then it might be possible under certain circumstances to determine the stability and the longevity of the $P = O$ isomer in the living plant by keeping a continuous check on respiration. In other words, a more exact answer must be found to the question as to which factors are responsible in the preparations applied in these tests for the increase in respiration.

In order to study these questions more closely, an examination was made of the effect of different substances constructed similarly to "Systox", and which were also available as separate $P = S$ and $P = O$ compounds.

In the first test series, the algae were placed in a 0.008 % emulsion of triethylphosphate or, alternatively, triethylthiophosphate. The substances are so slightly soluble in water that the concentrations could only be applied as emulsions. It was found that there was no influence whatsoever on the respiration or respiratory O_2 consumption in either case.

Since neither of these two compounds contain the thioether group so characteristic of "Systox", use was made in further tests of the dimethyl ester corresponding to "Systox".

One would expect a specific effect of the $P = S$ configuration also to occur here. As shown in Fig. 13, the respiration of *Ankistrodesmus* is increased following an addition of methyl "Systox", to about the same extent as when the corresponding "Systox" concentration is added. In contrast to "Systox", however, the O_2 consumption here not only increases in a solution or emulsion of the $P = S$ compound* but also in the same manner when the $P = O$ isomer is present. If consideration were to be given to a more or less specific difference between the effect of the $P = S$ and that of the $P = O$ isomer of "Systox", then it would have to be assumed that the mere exchange of both ethyl groups for the methyl group had given the $P = O$ compound — which so far had been ineffective — the effect of increasing respiration in this case, without influencing at the same time the efficacy of $P = S$ compound in any form whatsoever. Such an assumption is most improbable from the very outset. It would be much more to the point to assume that the methyl ester produced following a somewhat irregular synthetic process, like the $P = S$ diethyl ester, carries certain impurities, rearrangement products or by-products which are not present in the $P = O$ compound, and which might be held responsible for the increase in the consumption of O_2 .

The two "tri-esters", the triethyl phosphate and the triethyl thiophosphate, in particular, are the secondary constituent of "Systox" (verbal report of Dr. Schrader). The above-mentioned tests show that these two substances do not influence the gaseous metabolism even at a concentration which corresponds to the total "Systox" concentration. Consequently an effect which causes an increase in respiration cannot be considered by any means

* $P = S$ methyl "Systox" contained in the above case an admixture of 6% of the $P = O$ compound.

at the much lower concentrations which are used for mixing these compounds in the total preparation.

In addition to the admixtures of tri-ester, the $P=O$ isomer occurs in a high percentage as a rearrangement product* in the solutions of the $P=S$ compounds. The $P=S$ isomer which is richer in energy and less stable, changes to a certain extent in the solution during the course of several days into the $P=O$ form which is poorer in energy and more stable. One might

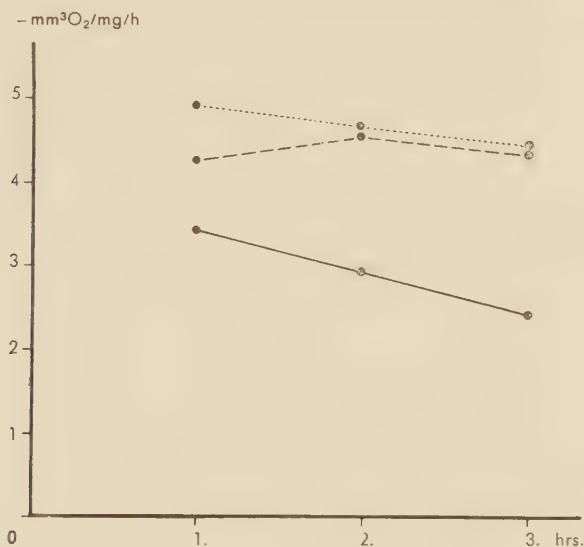


Fig. 13: *Ankistrodesmus*, respiratory O_2 consumption in a 0.01% "Metasystox" solution (+ emulsifier).

— Control
 - - - "Metasystox" $P=S$ isomer
 "Metasystox" $P=O$ isomer

Suspension density: 2.6 mg/5 ml. Direct KOH method.

expect, therefore, that the $P=S$ compound in a solution loses more and more of its ability to increase the respiration during the course of several days whereas a solution of the $P=O$ compound remains ineffective.

Therefore, in addition to fresh solutions, tests were also made on solutions or emulsions of the two isomers which had been left to stand up to 120 hours in a concentration of 0.01% at room temperature (pH 6.2). Analytical methods show that in solutions of this age, the $P=S$ form is changed to the thio compound to a high percentage (verbal report of Dr. Schrader). The effect which causes an increase in respiration is maintained here to a large degree,

* While this paper was in print, the author was informed of the results of new studies carried out by Metcalf et al., which were published in 1955 in the Journal of Economic Entomology. According to these results, the phosphate sulfoxides and sulphones or, alternatively, the corresponding thiophosphate compounds are present in "Systox" solutions as rearrangement products. In order to clarify the problems discussed here, such substances should also be included in the investigations.

and corresponds to the effect of a freshly prepared solution. Consequently a change of the $P = S$ form to the $P = O$ form does not involve any considerable decrease in the effect on respiration.

In the experiment illustrated in Fig. 14, the algae were placed in $P = S$ and $P = O$ solutions of different ages. During these tests, the effect of the $P = S$ compound did not weaken for more than 40 hours. However, it was

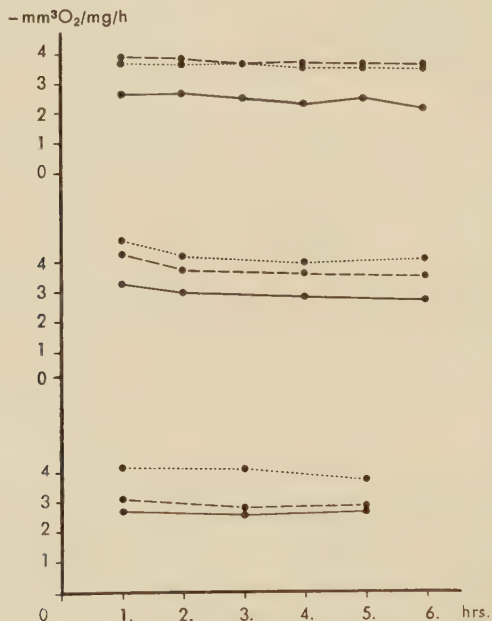


Fig. 14: *Ankistrodesmus*, respiratory O₂ consumption in 0.0065% "Systox" solutions of different ages.

Age of each solution at the start of the experiment :

Top : 35 hrs. Centre : 25 hrs. Bottom : 1 hr.

— Control

- - - Algae in "Systox" $P=O$ solution

..... Algae in "Systox" $P=S$ solution

Suspension density : 2.2 mg/5 ml. Direct KOH method.

clearly evident that after only 22 hours the algae in the emulsion of the $P = O$ isomer also had a much higher consumption of O₂ than the cells in the control solution. After a period of approximately 35 hours, the respiratory consumption of O₂ in the emulsions of the $P = S$ and the $P = O$ form reached a level above the control which was equal for both forms. The result corresponds largely with the curve plotted for the experiment illustrated in Fig. 9. In the latter, the differences between respiration in the control and respiration in the $P = O$ isomer were the same after about 25 hours. It would not have been possible in this case to make any definite assertions in view of the low absolute values of the respiration.

Since it was not possible earlier to make a certain configuration in the molecule of the $P = S$ compound responsible for the effect which causes an increase in respiration, it seems all the more probable following the last experiment (Fig. 14) that the increase in respiration can be attributed to rearrangement or by-products. It might be thought that a substance which only acts slightly on the respiration, will first cause the occurrence of the full effect synergistically, in the presence of a small quantity of another compound. Such synergisms and activator effects are not unknown with regard to the animal physiological behaviour of insecticides, and at times are not without significance (Cox, Incho and Greenby, Mayer et al. etc.). It might also be assumed that the differences in the physiological effect are due to a different state of division between the two emulsified substances. If so, it would also have to be assumed that the emulsions, due to the very different solubility of the isomers, each age to a different degree under certain circumstances, and that, therefore, the absorption processes in the two emulsions change in different ways.

Our experience would suggest that the first assumption, namely the occurrence of certain rearrangement phenomena, provides the more natural explanation for the effects discussed here. This assumption that rearrangement phenomena actually do occur is not the outcome of an ad hoc hypothesis but is based on similar indications arising out of a number of other results (see Page 315).

3. Evolution of CO_2 in the presence of high "Systox" concentrations

Previous manometric experiments had revealed that the consumption of O_2 is increased by "Systox". Moreover, the consumption of O_2 has always been regarded as a reliable standard for determining the intensity of respiration. However, the possibility cannot be ignored that the increased consumption of O_2 indicates a stimulation of other oxidation processes in the examined culture.

In particular, consideration must be given here to oxidation processes which go on in the case of sub-lethal damage being caused to the cells.

In order to make sure that the increase in the consumption of O_2 is a genuine increase in the respiration, the production of CO_2 was also determined in addition to the O_2 consumption. For this purpose the $\pm$ KOH method mentioned in the formula appendix and in the section allotted to experimental methods, was applied in the following experiments.

In a number of tests which proceeded similarly to Test 15, it was shown that an increase of the O_2 consumption in the case in question is always accompanied by a corresponding increase in the production of CO_2 . In other words, there is a genuine increase in the consumption of carbohydrate by respiration. These tests will be discussed in more detail at a later stage with respect to other factors (see Page 305).

4. Reversibility of increase in respiration

Large and persistent increases in respiration are normally effects which are undesired in practice, insofar as they are not combined with a simultaneous stimulation of the processes of synthesis. Whenever an increase in respiration occurs in our case following an excessive dosage of preparation,

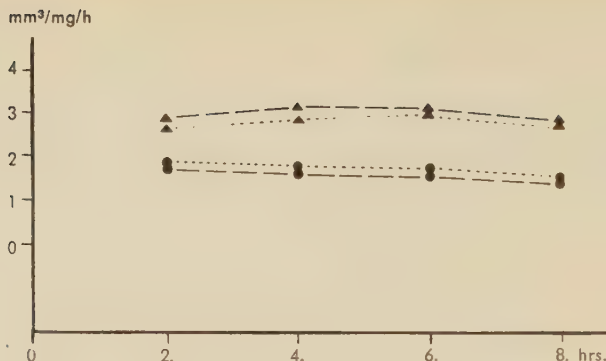


Fig. 15: *Ankistrodesmus*, O₂ absorption and CO₂ evolution in a nutrient solution with a 0.01 % content of "Systox".

● ● Control
 ▲ ▲ Algae in "Systox" solution
 --- O₂ absorption
 CO₂ production

Suspension density: 14.0 mg/6 ml. Age of culture: 9 days. $\pm$ KOH method.

it is important to know whether a dilution, washing out or translocation of the preparation will soon lead to the respiratory processes becoming normal again. Therefore, a check had to be made whether, and if so to what extent, the increase in respiration observed here is reversible.

The algae of a 7 day old suspension were transferred in the usual manner to a nutrient solution with a 0.01 % content of "Systox". At the same time, the algae required for the control experiment were placed in a fresh nutrient solution free of preparation. The cells were left in these solutions for five hours. During this time, the cultures were shaken in the culture flasks in the usual manner, and illuminated with approximately 800 lux. A pre-illumination of this kind is desirable, otherwise the algae will be in a state of starvation very soon after the start of the subsequent manometric measurements, and the absolute values for respiration will then be really low.

After the cells had been in the "Systox" solution for five hours, one half of the suspension was washed three times in the centrifuge with "Systox"-free nutrient solution, and then transferred to a freshly prepared "Systox"-free nutrient solution (2). The other half was also centrifuged three times but was always put back in a "Systox" solution (1). The controls were treated in a corresponding manner, and transferred to fresh nutrient solution free of preparation (3). Following this pre-treatment, the respiration of the three suspensions was determined by means of the KOH method. Fig. 16 illustrates the results of this experiment.

Whereas the respiration of suspensions 1 and 2 remained more or less at the same level for the first two hours, the respiration increase in suspension 2 dropped slightly in comparison with suspension 1 from the 4th hour onwards. After 9 hours, the suspension from which the preparation was washed out, showed a 50 % smaller increase in respiration than the algae which were transferred to the "Systox" solution. After 13 hours, the increase in respiration

can no longer be proved with certainty for suspension 2. It is, therefore, obvious from the experiment illustrated in Fig. 16 that the respiration increase is not so great after the preparation has been washed out of the algae.

The "Systox" determinations with the aid of a ^{32}P labelled preparation discussed on Page 281 show that after the algae have been left in a "Systox"-free solution for five hours, in other words corresponding to a five-hour manometric measurement, more than half the ^{32}P established at the beginning of the test is still present in the cells. The slow decrease of the respiration rate corresponds to the slow disappearance of the labelled compound from the algae. It should be considered here that according to past experience, any effective rearrangement products will also contain the labelled P atom, and are thus included in the tracer measurement.

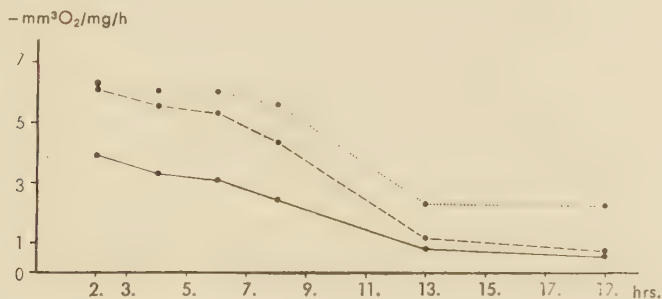


Fig. 16: *Ankistrodesmus*, reversibility of the increase in respiration caused by "Systox".

- Algae in "Systox" solution for 5 hours, then for a further period in "Systox" solution, concentration of applied solution 0.01 % P=S isomer. Suspension density: 7 mg/8 ml. Direct KOH method.
- Algae in "Systox" solution for 5 hours. "Systox" washed out, then for a further period in "Systox"-free test solution.
- Control, algae in normal solution, centrifuged three times, then for a further period in a normal solution.

5. Effect of "Systox" on the respiration and the synthetic efficiency of *Ankistrodesmus* and *Chlorella* in a glucose-containing nutrient solution

The respiratory consumption of O_2 by algae is multiplied by adding glucose to the nutrient solution. Only a small part of the sugar given is oxidized by the cells while the remaining glucose is incorporated. The oxidation provides the energetic basis for the simultaneous glucose assimilation (Myers 1947).

The experiment illustrated in Fig. 17 shows the respiration of *Ankistrodesmus* in a 1 % glucose solution (Glucose Hydrate "Bayer"). As soon as the glucose is added, the respiration immediately begins to increase steeply, and then following a somewhat more gradual rise of the curve, it reaches its maximum value, approximately 900 % of the basic rate of respiration, at the end of 3 hours.

Once glucose has been added, the respiration in the "Systox" solution increases to several times the basic rate of respiration just as in the control. It is noticeable here, however, that the respiration with "Systox", which in a pure inorganic nutrient solution is at first above the control value, remains well below the curve of the control experiment after glucose is added. The increased respiration caused by the addition of glucose is, therefore, somewhat

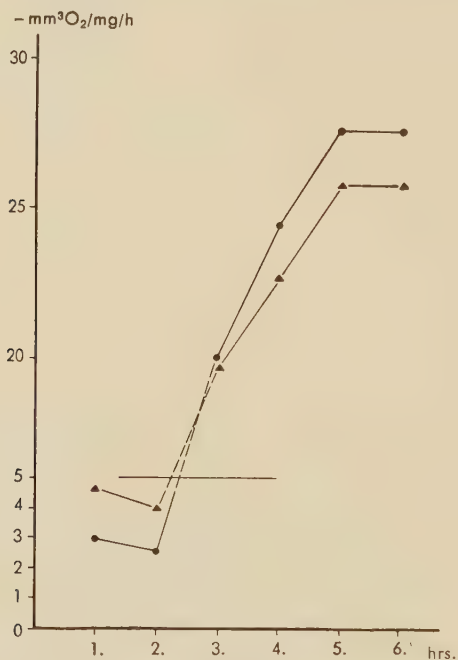


Fig. 17: *Ankistrodesmus*, respiration in 0.006% "Systox" solution. In inorganic solution for 1st and 2nd hour. After the 2nd hour, glucose was added until the glucose content of the solution amounted to 1%.
 ● ● Control
 ▲ ▲ Algae in "Systox" solution
 Suspension density: 3.5 mg/5 ml. Direct KOH method.
 Ordinate shortened between 5 and 20.

restricted during the course of the test in the presence of "Systox". Three other measurements produced results similar to those obtained in the experiment illustrated in Fig. 17.

Because no conclusions can be drawn from this experiment as to the extent of the glucose assimilation, and as no data can be given on the ratio of oxidation to assimilation, a method has been employed in the following experiment which permits exact data to be given on this ratio.

In the experiment illustrated in Fig. 18, the respiration was kept under observation after the addition of glucose up to the point where the O_2 consumption of the cells dropped to the value of the basic respiration following

the complete incorporation or consumption by respiration of the applied sugar. At the same time, a parallel test was conducted to determine the respiration in a pure inorganic nutrient solution.

When carrying out such a determination which must cover the entire period of the increased glucose respiration, it is advisable only to add a small quantity of sugar to the suspensions. In addition, it is also commendable to select an algal strain which removes the added sugar as quickly as possible from the solution by incorporation or oxidation. According to the experience of the Marburg Botanical Institute, a particularly suitable organism proved to be a *Chlorella* strain which is registered under the number 211/11 h in the Pringsheim collection.

The percentage of O_2 consumption resulting from the oxidation of the glucose is obtained by subtracting the entire quantity of oxygen consumed by respiration in the inorganic solution (endogenous) from the corresponding values for respiration in the glucose solution. Then the quantity of oxidized or assimilated glucose can be determined by means of a single calculation.



Fig. 18: *Chlorella* "11 h", respiratory consumption of O_2 in a 0.01% solution of "Systox". Each measuring point gives the quantity of O_2 consumed in mm^3 as the average of 3 measurements/vessel/30 minutes.

▲ ▲ Control
● ● Algae in solution containing "Systox"

Top curve: Addition of glucose 45 minutes before start of test ($45 \mu\text{mol}/5 \text{ ml}$)

Bottom curve: Algae during the test in inorganic nutrient solution

Suspension density: $8.5 \text{ mg}/5 \text{ ml}$. Age of culture: 6 days. Direct KOH method.

The dashed-line part of the curve was determined by extrapolation.

6 mol of O_2 are used up in consuming 1 mol of glucose by respiration. When the molar volume for 24°C is calculated at 24.38 l according to $V_t = V_o (1 + \alpha t)$, then $6 \times 24.38 = 146.28 \text{ l}$ of oxygen are used up in the consumption of 1 mol. of glucose by respiration. Consequently 146.28 mm^3 of O_2 are required for the oxidation of 1 micromol.

The percentage of sugar consumed by respiration ought to increase when the synthetic process in the cell is disturbed by "Systox". More glucose would then be consumed than is normally necessary for the incorporation of carbo-

hydrate. This experiment also showed at first that the increased glucose respiration in "Systox" solution, just as in the case of *Ankistrodesmus*, was well below the values of the control whereas the endogenous respiration increased by approximately 100 % as expected. In the end, however, the respiration of sugar in the "Systox" solution also reached the corresponding maximum value of the control experiment. In principle, it also appears possible by adding glucose for the consumption of O_2 to increase in a "Systox" solution to the same extent as in the nutrient solution without preparation. The delayed increase in respiration in the presence of "Systox" was explained by a change in the permeability. Apparently there is never any increase in the additional respiration caused by the addition of "Systox" in an inorganic solution. The additional glucose respiration is not increased by the preparation.

There is one other peculiarity which becomes noticeable when the course of the curve is studied. Once the additional respiration has decreased, the O_2 consumption in both curves of the experiment is still well above the values of the algae which were examined simultaneously in a pure inorganic solution. The explanation for this difference is that in an inorganic nutrient solution, the absolute value of respiration decreases proportional to the consumption of the respiration substrate. On the other hand, the algae showed a noticeable increase in respiration substance during the seven hours they were kept in the glucose solution due to the increased carbohydrate and nitrate assimilation. This respiration substrate has the same value both in the presence and in the absence of "Systox".

In order to draw a conclusion on the progress of the synthetic processes in the cells from the extent of the respiratory consumption of O_2 , use is made of the formulae referred to earlier (cf. Page 295). From the additional respiration of glucose it is possible to calculate the quantity of oxidized glucose, and then by subtracting this value from the total glucose the quantity of incorporated sugar can be computed. (For technical reasons, the dashed-line part of the curve had to be determined by extrapolation.)

If 146.28 mm^3 of O_2 are required for the oxidation of 1 micromol of glucose, as shown above, then a total of $6,582 \text{ mm}^3$ of O_2 should be absorbed by the algae following the addition of 45 micromol when the sugar is completely consumed by respiration:

When the normal endogenous respiration (respiration value of the suspensions in inorganic solution) is subtracted in this experiment from the total respiration of the glucose suspensions, the following values are obtained from the time of the first measurement until the curves drop to the normal value:

Table 6. Percentage of the normal endogenous respiration and the additional respiration of the glucose in the total respiration of *Chlorella* cells in a 0.01 % "Systox" solution with glucose present in the nutrient solution. Suspension density: 8.5 mg/5 ml; age of culture: 6 days.

	Control	Algae + Preparation
Total respiration ($-\text{mm}^3 O_2$)	1,055	1,169
Endogenous respiration	101	189
Additional respiration of glucose	954	980

During the course of the experiment until the additional respiration ceased, 954 mm³ of O₂ were consumed in the control for the oxidation of the applied glucose, and 980 mm³ in the presence of "Systox". In contrast to the assumption usually made in the past, it is essential for the purpose of these calculations that the basic respiration continues unchanged in the presence of glucose. Results obtained by Daniel, which will be published elsewhere, justify this assumption. When approximately 6,580 mm³ of oxygen per vessel are required for the oxidation of the entire glucose, then 14.5 % of the applied glucose will have been oxidized by the algae in the normal solution, whereas the algae in the "Systox" solution will have oxidized 14.8 % of the applied sugar. The slight difference between these two values lies within the limits of errors. The ratio of sugar consumed by respiration to the incorporated material, and the absolute quantity of incorporated glucose are two factors which apparently do not change due to the addition of "Systox". It is merely the absorption of glucose in the presence of the preparation which seems to be delayed somewhat.

The synthetic processes which are combined with the incorporation of glucose, apparently proceed normally even following the addition of "Systox". This result conforms with the observations already mentioned earlier (cf. Page 280). The growth of plasm does not stop at first after *Ankistrodesmus* is transferred to a "Systox" solution of this strength.

6. Respiratory consumption of O₂ by roots in a "Systox" solution

A certain interest is shown in the measurements of respiration on the roots of higher plants when discussing the effect of systemic insecticides on metabolism. Wherever the prevailing conditions demand it, systemic preparations are also applied to the roots by sprinkling or by means of capsules. The results of the cress test and the studies made on the absorption of water by roots from a "Systox" solution (Tietz) give rise to the expectation that the physiological processes in the root may also be influenced by the preparation. Moreover, roots are particularly suitable for manometric measurements for methodical reasons. They are the only tissues of cultivated plants on which manometric determinations can be carried out by the usual methods without having to place the test objects in surroundings where there are non-physiological conditions.

The roots of the *Tradescantia* cutting selected for the test were cut off with razor blades two hours before the start of the experiment, and placed in freshly prepared Knop's nutrient solution. Later these roots were transferred to the test vessels by means of a soft brush. In all cases, the preparation was added during the test as an emulsion from the side arm on the main compartment. It had been made certain beforehand in control experiments that the emulsifier does not act on the roots. The direct KOH method was employed for carrying out the measurements. After the manometric measurements, the dry weight of the roots put into each vessel was determined.

The respiratory consumption of O₂ by the *Tradescantia* roots immersed in the solution of preparation was the same as that for the roots in the control solution up to a concentration of 0.01 % "Systox" commercial preparation. The measurements were continued for 20 hours after the addition of the preparation. Sugar beet roots did not show any signs of disturbance even at a concentration of 0.08 % "Systox".

In further tests, the O_2 absorption by the *Tradescantia* roots was measured in more highly concentrated solutions of preparation. The first disturbances were observed at concentrations between 0.05 and 0.07 %. The respiration decreased considerably after the roots were transferred to a 0.08 % solution (Fig. 19). At this concentration irreversible damage is caused to the roots after about only 5 hours. These injuries are clearly perceptible also upon microscopic examination. Similar injuries were first caused to the sugar beet root at a concentration of 0.1 % commercial preparation in the test solution. It is most surprising that no increases in the consumption of O_2 were established in any of the measurements of respiration on the roots, increases which were so characteristic of the green tissues (*Helodea* leaves, *Chlorella*, *Ankistrodesmus*) examined in the "Systox" solution.

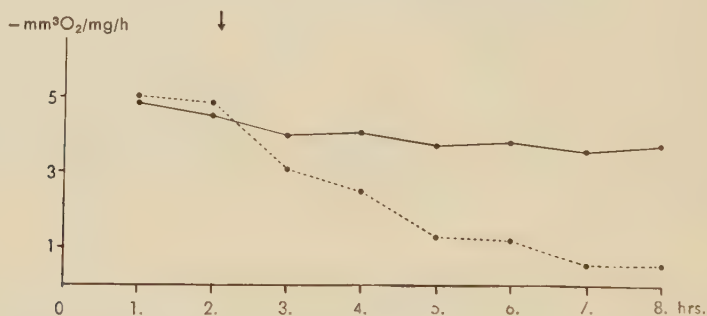


Fig. 19: Respiration of *Tradescantia* roots.

— Control
 After the 2nd hour (arrow), addition of "Systox" up to a "Systox" content of 0.08% in the test solution.

Direct KOH method.

Certain disturbances may, therefore, be caused when higher concentrations act on the cells, as this finding shows. Similar injuries to leaves (Tietz), known to the practitioner as "scorch" caused by excessive dosages, have often been reported also following the application of other systemic insecticides (e. g. "Schradan" preparations). Ripper et al. point out, for example, the relatively high sensitivity of *Vicia faba* to "Schradan", and mention considerable disturbances with certain apple varieties (Cox Orange Renette, Pippin American, Worcester parmaene).

According to past experience, injuries of a general nature must be expected when solutions containing approximately 0.1 % "Systox" are in lengthy and direct contact with the root system. For the same reason, the conventional application of several systemic products which are highly toxic to warm-blooded animals, by burying them in water-soluble capsules which contain the undiluted preparation (Hanna) appears to be particularly contestable.

The roots were always placed in the aqueous solution or emulsion of the preparation in the tests described in this paper. It is, however, a well-known fact that "Systox" and other systemic preparations too, are absorbed from the

soil to a lesser degree than from nutrient solutions. Moreover, it was known that the absorption of "Systox" (Tietz), and also the absorption of "Schradan" (David, Casida et al.), depends to a great extent upon the type of soil from which the preparation is to be absorbed. In order to obtain exact data on this, an attempt was made to record the adsorption of the preparation in soil layers of various depths and different composition.

The tests produced very definite results. It was found that the content of preparation (or the content of ^{32}P) decreased by one power of ten after passing through a layer of approximately 4 cm of greenhouse soil, or 6 cm of loam, or 8 cm of sand.

In view of these results, it is most improbable that the application of "Systox" by means of the soil watering technique in the region of roots will be followed by the development of concentrations in the capillary water which will cause perceptible root injuries. Concentrations of more than 0.08 % commercial preparation, which harmed the roots of *Tradescantia* and sugar beet, are not normally applied. Concentrations which have an inhibiting effect in the cress test, do not normally occur in the region of the root either, and if they do then they are reduced very rapidly by dilution.

During the course of time, the concentration of the preparation in the capillary water of the soil decreases more and more as the result of washing out and other forms of translocation. The strongly absorptive "Systox" molecules on the soil particles are then constantly given off again into the capillary water. The good absorbability of "Systox" in the soil, therefore, not only prevents the occurrence of phytotoxically effective concentrations but at the same time it is also one of the reasons for the good persistent property of preparation following application by means of the soil watering technique.

D. Measurements of Photosynthesis

1. Influence exerted by different "Systox" concentrations on photosynthesis

Several exploratory tests were first carried out to examine the effect of low concentrations of "Systox" and "Schradan" on photosynthesis. Several of these tests are summarized in Table 7.

Table 7: Photosynthesis of *Ankistrodesmus* in "Systox" and "Schradan" solutions. One-vessel method, 2.4 mg dry weight/8 ml. Age of culture: 5 days. The given values are the averages of five successive hourly readings.

	"Systox" active substance			"Schradan"		
	Control	0.001 %	0.004 %	Control	0.006 %	0.016 %
+ mm ³ O ₂ /mg/h	194	191	178	193	190	160

Neither of the two preparations tested here had any influence on photosynthesis in the low concentration ranges. The photosynthetic quotient (PQ) of the algae in a solution of this concentration was also determined by means of the two-vessel method in subsequent experiments. The quotients did not

deviate from the control even in the presence of preparation. After reviewing previous findings in which there was no influence on respiration at "Systox" concentrations of up to 0.005 %, it may, therefore, be said that the examined unicellular organisms in solutions containing less than 0.004 % "Systox" do not differ in any way whatsoever from the cells in the preparation-free control solution as regards their basic processes of metabolism.

The same was also established with the *Helodea* leaves which were examined by the same method. These results conform with the values obtained in the dry weight determinations (Fig. 4).

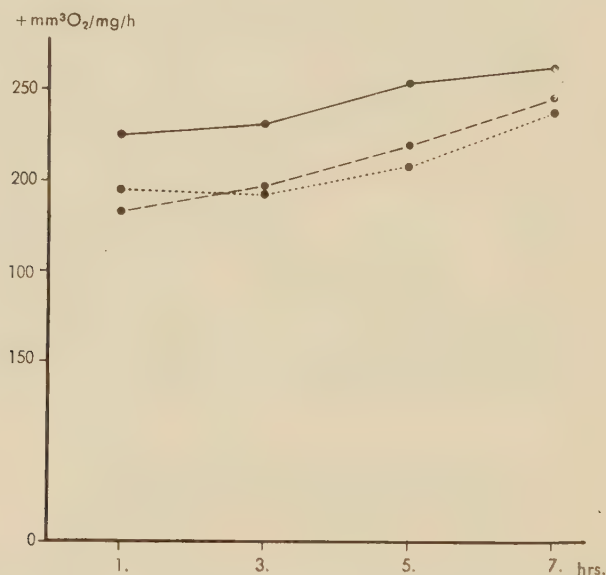


Fig. 20: *Ankistrodesmus*, photosynthesis.

— Control
 --- Algae in 0.007% "Systox" solution (P = S)
 (+ emulsifier)
 Suspension density: 1.6 mg/5 ml. One-vessel method
 Algae in 0.007% "Systox" solution (P = 0)

Just as in all previous tests, the higher concentrations (from 0.004 % onwards) of the preparation had to be applied as emulsions for the photosynthetic measurements. too. Once again a check was first made to ascertain that the pure emulsifier does not have any influence on the metabolism processes being examined.

From the two experiments summarized in Table 7, it is evident that "Systox" and "Schradan", starting at a certain limit concentration, weakly inhibit the photosynthesis of the objects examined here. This limit will be approximately 0.004 % for "Systox", and a little over 0.01 % for "Schradan". Once again, only the concentrations of equal insecticidal potency (cf. Page 276) are comparable, and not the absolute values of the concentration of the preparation.

The effect of more highly concentrated emulsions was examined in further tests. It was established that the two isomers of "Systox", at correspondingly high concentrations, inhibit photosynthesis to exactly the same extent (Fig. 20), whereas they differ greatly in their effect on respiration (cf. Page 286). This difference is probably due to disturbances of photosynthesis and increases in respiration being caused by different factors.

The inhibition of photosynthesis began very soon after the addition of the preparation. In this experiment, the preparation was added 40 minutes before the start of the measurement. Other measurements of photosynthesis were carried out up to 45 hours after the addition of the preparation. In the long-term tests, the algae were first shaken for 35 hours (just as in the tests

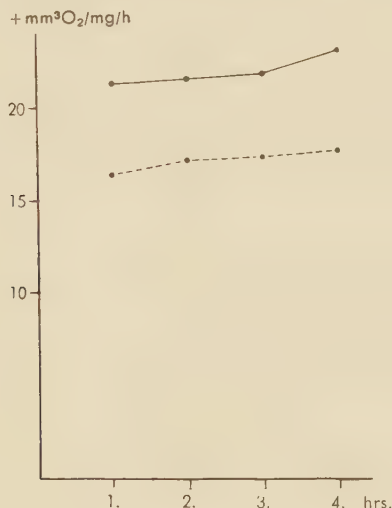


Fig. 21: *Helodea canadensis*, photosynthesis, 0.007 % "Systox" solution (+ emulsifier).

— Control
 - - - Leaves in "Systox" solution. One-vessel method.

described in Page 292) in weak light (800 lux) at 20 ° C without passing through a mixture of air and CO₂. After "Systox" (0.007 %) had acted for such a long period, the measurements produced exactly the same picture as that observed immediately following the addition of the preparation. Therefore, the disturbance caused at this concentration is still not very serious (for contrast, cf. Page 304).

Just as in the case of respiration, the inhibition of photosynthesis is exactly the same for *Helodea* as for *Ankistrodesmus* (Fig. 21). Very soon after the transfer to a 0.007 % active substance solution or emulsion, a 20 % inhibition of the photosynthetic gas exchange is caused.

In comparison with Fig. 20, an illustration is given below of a photosynthetic experiment with *Ankistrodesmus* in a 0.016 % solution of "Schra-

dan" (Fig. 22). The relatively low suspension density and the low age of the culture explain the higher photosynthetic values, also in the control.

The photosynthesis is strongly inhibited from the beginning of the experiment onwards. The inhibition of approximately 25 % and its consequences are also made manifest by the fact that in contrast to the control, the photosynthesis in the "Schradan" solution hardly increased to any noticeable extent during the six hours the test was run.

On comparing the experiments of Figs. 11 and 20, it is seen that the depression of the apparent photosynthesis cannot be explained by the simul-

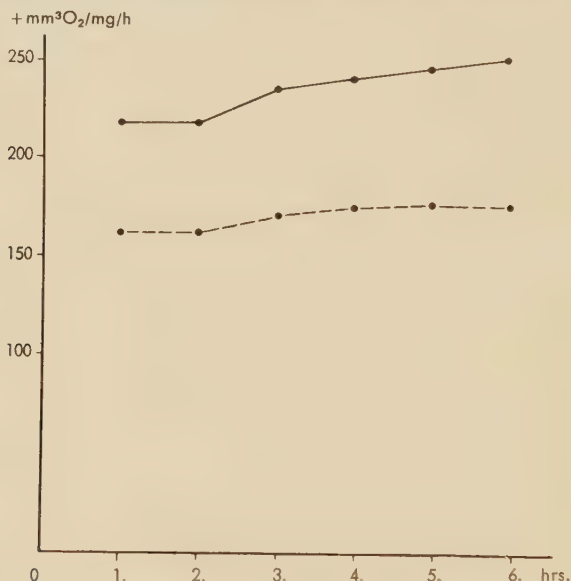


Fig. 22: *Ankistrodesmus*, photosynthesis in a 0.016 % "Schradan" solution (+ emulsifier).

— Control
 --- Algae in solution of preparation
 Suspension density: 2.4 mg/5 ml. One-vessel method.

taneous increase in respiration. Whereas the additional respiratory consumption of O₂/5 hrs./mg amounts to about 3 mm³, the O₂ production in the photosynthetic test is almost 80 mm³ less in the presence of "Systox". 5 hrs./mg than in the control. The depression of the O₂ production in light following the addition of "Systox" is, therefore, to be attributed to an inhibition of the real photosynthesis.

2. Reversibility of the photosynthetic disturbances

It was illustrated above that the increase in the respiratory consumption of O₂ drops again following a reduction of the influential concentration of preparation. Consequently, the harm caused cannot be of a very serious nature. In the following, the influence of the preparation on photosynthesis will be further analyzed and, in particular, it will be established by way of com-

parison to what extent the depression of the photosynthetic processes is also reversible.

Just as in the study made on the reversibility of respiration disturbances, three differently pre-treated algal suspensions were examined here, too, in conformity with the experiment described on Page 292:

1. Algae in a "Systox" solution for five hours, then in a "Systox" solution for a further period.
2. Algae in a "Systox" solution for five hours, preparation washed out, then in the "Systox"-free solution for a further period.
3. Control algae exposed to all processes just as groups 1 and 2, but in a "Systox"-free nutrient solution in each case.

Four vessels were filled with each suspension. The manometric measurements were started one hour after the transfer to the test solutions.

There are definite differences between the curves of Fig. 23 and Fig. 16. Whereas the respiration at first returns gradually to the normal value over a period of more than 12 hours after the algae have been washed several times, the photosynthetic efficiency of the algae immediately after "Systox" is washed out more or less corresponds to the values of the control. However, a weak depression is maintained and this remains unchanged for some considerable time, whereas the photosynthesis of the algae which are left in the

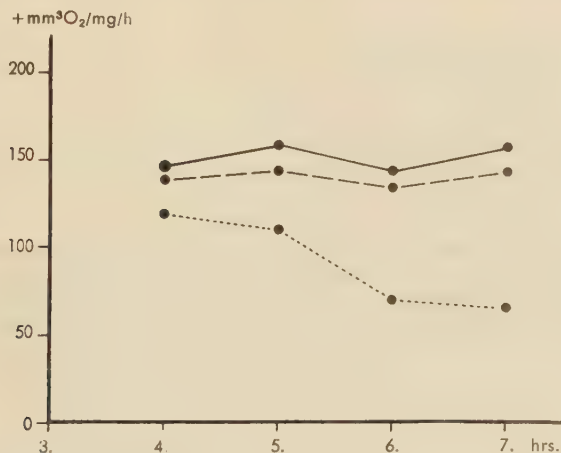


Fig. 23: *Ankistrodesmus*, reversibility of the photosynthetic depression caused by the addition of "Systox".

- Control, algae exposed to all processes like the other two groups, but in "Systox"-free nutrient solution in each case.
- - - Algae in "Systox" solution for five hours, preparation washed out by centrifuging three times, then in "Systox"-free solution for further period.
- Algae in "Systox" solution for five hours, then left in "Systox" solution for further period.

Suspension density: 1.3 mg/5 ml. One-vessel method.

Only the first measured value of curve 1 is comparable with curves 2 and 3 because in test 1 the algae were constantly subjected to further injuries as the result of being left in the solution of preparation.

highly concentrated emulsion (0.01 % active substance), continues to decrease. This phenomenon is probably due to secondary photooxidative injuries which, according to the experiment illustrated in Fig. 20, are not caused following an application of low concentrations of active substance (0.007 %). The difference between sample 1 and sample 2 was equal in the two parallel tests, although it was always so slight that it could not be recorded with certainty.

In order to prove that the photosynthetic test can be compared with the respiration measurements of Fig. 16, the respiratory consumption of O_2 was determined for the same suspensions at the end of the photosynthetic experiment. The measurement of respiration was made 9 hours after the transfer of the algae to the test solution. The results are given in Table 8. They fully conform with the values of the experiment illustrated in Fig. 16. The respiration in this case, therefore, appears to be similar to that shown in Fig. 16, whereas the photosynthesis takes a completely different course from the curves of this respiration experiment.

Table 8: Respiratory consumption of O_2 by the *Ankistrodesmus* suspensions at the conclusion of the photosynthetic measurement in the experiment illustrated in Fig. 23 (9 hours after the start of the test), direct KOH method.

	— $mm^3 O_2/mg/h$
Algae in "Systox"-free solution	6.0
Algae transferred from "Systox" solution to solution free of preparation	8.7
Algae left in 0.01 % "Systox" solution	11.5

A considerable part of the preparation is removed from the cells after it is washed out three times (Table 3). This explains why the inhibition of photosynthesis has almost completely ceased at the start of the manometric measurement. Even a further spell in "Systox"-free solution, in which more than one third of the remaining preparation is washed out (Table 3), does not cause the photosynthetic curve to rise any more. It must be assumed that the photosynthetic value has almost returned to normal again immediately after centrifuging.

On the other hand, the respiration is still at the same level after three washings as in a fully concentrated "Systox" solution. After a further spell in preparation-free solution combined with repeated washing out of the preparation from the cells (cf. Table 3), the respiration, too, gradually returns to its normal value. These findings disclose three possibilities for explaining the occurrences:

1. The respiration is stimulated so persistently that the increase continues for a limited period even after the "Systox" has been washed out, while in contrast the photosynthetic depression becomes reversible in a very short time.
2. It is very difficult to wash out the active ingredient molecule from the regions of intracellular structure where it acts on the respiration, yet it is easy to remove from those parts where it gains influence on photosynthesis.

3. In contrast to the photosynthetic depression, the increased rate of respiration may be attributed to secondary or by-products which take more time to remove from the cells than the original preparation.

This conception agrees with several other findings put forward in this paper. It is most probable that all three possibilities will have to be considered together in order to explain the test results of Figs. 11 and 23.

E. Nitrate assimilation (quotients of gas exchange)

In addition to photosynthesis and respiration, there are several other factors influencing the state of the quotients which can be recorded by determining the quotients* of the gas metabolism with the indirect two-vessel methods. The size of the CO_2 deficit or, alternatively, the amount of extra carbon dioxide formed depends chiefly upon the oxidation stage of the respiration substrate and upon the extent of the synthetic processes including the nitrate assimilation.

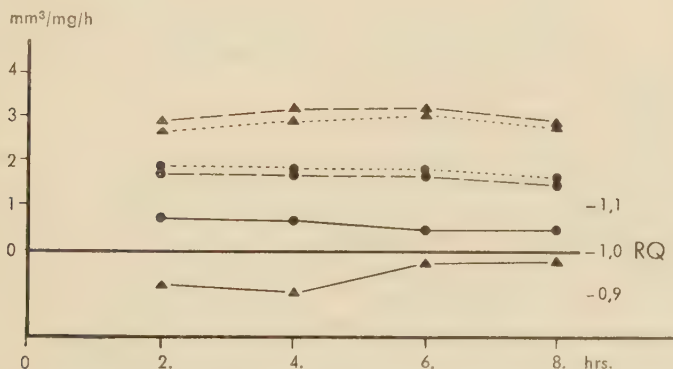


Fig. 24: Effect of the addition of "Systox" on the respiratory consumption of O_2 , CO_2 production and respiratory quotients for *Ankistrodesmus* in nitrate solution.

- ● Control
- ▲ ▲ Algae in 0.01% "Systox" solution
- O_2 consumption
- CO_2 production
- Respiratory quotient

Suspension density: 14.0 mg/6 ml. Age of culture: 9 days. $\pm$ KOH method.

1. State of the respiratory quotients in the presence of "Systox" in a nitrate solution

In order to prove that the increase in the respiratory consumption of O_2 caused by "Systox" represents a genuine increased rate of respiration, the CO_2 production was determined above by means of the $\pm$ KOH method. Apart from the importance of this experiment with respect to the special formulation of the question, it also supplies other valuable information.

* RQ refers to the ratio $-\text{CO}_2/\text{O}_2$, and PQ to the ratio $-\text{O}_2/\text{CO}_2$. To simplify the explanation, the negative sign of the quotients is not taken into consideration. Therefore, when the quotients change from 0.9 to 1.1, reference is made to a quotient increase.

Fig. 24 again illustrates the experiment already described on Page 292. In addition, the values of the RQ are entered in Fig. 24. Table 9 also includes the extra CO₂ values for this experiment as well as the quotients.

Table 9: *Ankistrodesmus*, RQ and extra CO₂ production of algae in a 0.01 % "Systox" solution. Suspension density: 14.0 mg/6 ml. Age of culture: 9 days. $\pm$ KOH method.

	Extra CO ₂ /mg dry weight/h.		RQ	
	+ Preparation	Control	+ Preparation	Control
1st hour	— 0.26	+ 0.12	0.91	1.07
2nd hour	— 0.32	+ 0.10	0.90	1.06
3rd hour	— 0.07	+ 0.07	0.98	1.04
4th hour	— 0.06	+ 0.07	0.98	1.04
Total:	— 0.71	Total: + 0.36	Average: 0.94	Average: 1.05

The quotient of the control remains at approximately 1.05 throughout the whole test, caused by the above-mentioned factors. In the presence of "Systox", the extra CO₂ production not only ceases, but is in fact smaller than the O₂ consumption. Consequently the quotient is between 0.9 and 1.0.

In addition to the lack of nitrate assimilation and a depression of the corresponding synthetic processes, the occurrence of oxidation processes outside the respiration, which are probably caused by the action of injurious concentrations of the preparation, might be held responsible for the high consumption of O₂ and the reduction of the quotients to less than 1. Such an assumption is countered, however, by the reversibility of the effect causing an increase in respiration proved above, which renders it impossible for the harm inflicted to become serious.

2. Measurements of respiratory quotients in nutrient solution with ammonium salt as the source of nitrogen

Several determinations of the quotients in nitrate-free solution were carried out to establish whether the observed quotient changes are to be attributed to disturbances of the nitrate reduction or to an inhibition of other synthetic processes influencing the gas metabolism.

Just as in the nitrate solution, the RQ is immediately below 1 (Fig. 25) in the ammonium solution when "Systox" (0.01 % active substance) is present. The O₂ absorption is greater than the CO₂ production, even though only very slightly, throughout the whole experiment. The quotient is greatly depressed in the ammonium solution particularly during the first few hours of the experiment, just as it is in the nitrate solution.

On the other hand, the quotient of the control is somewhat lower than in the nitrate solution but just the same it is well above 1 despite the elimination of the nitrate reduction. Syntheses of carbon compounds with low oxidation levels may be responsible for this formation of extra carbon dioxide. On the basis of elementary analyses with normally fed *Chlorella*, Myers (1949) calculated quotients of up to 1.1 in ammonium solution, which he explained as being due solely to such syntheses.

Therefore, it is evident that "Systox" inhibits not only the nitrate reduction but also other synthetic processes. It is most interesting that this inhibiting effect is produced at the same concentrations at which the preparation causes an increase in the respiratory processes. An inhibition of the syntheses is also caused in a similar manner by 2.4-dinitrophenol which at the same time, in a certain concentration range, also has the effect of increasing the rate of respiration.

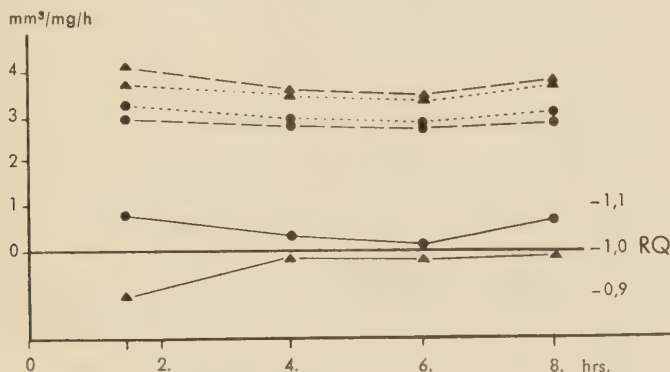


Fig. 25: Effect of "Systox" on the respiration of *Ankistrodesmus* in nutrient solution with ammonium salt as the source of nitrogen.

- ● Control
- ▲ ▲ Algae in 0.01% "Systox" solution
- Respiratory quotient
- O₂ consumption
- CO₂ production

Suspension density: 12.5 mg/6 ml. Age of culture: 5 days. $\pm$ KOH method.

3. State of photosynthetic quotients in the presence of "Systox"

Just as it is customary to take a respiratory quotient of 1 as the basis, the O₂ production of the photosynthesis is also calculated in most cases by means of the direct two-vessel method, assuming a PQ to equal 1.

According to the experience of Pirson et al. and Krollpfeiffer, the quotient is expected, however, to increase well above 1 (between 1.0 and 1.2), particularly with young cultures which have a relatively high nitrate assimilation. The calculation of the O₂ production by means of such a measurement based on a PQ of 1, consequently gives most unreliable values. Both the absolute values and the progress of the O₂ production may prove to be considerably incorrect.

In order to avoid these errors, the O_2 production and CO_2 consumption in photosynthesis were determined in several tests in a 0.1 % "Systox" solution by means of the vessel difference method (see formula appendix) *.

Fig. 26, just like earlier tests, again shows the considerable depression of the photosynthesis in a 0.01 % emulsion of "Systox" active substance which, therefore, also occurs when the O_2 production is determined by means of the two-vessel method. In this case, the PQ remains constant at approximately 1 in the presence of the preparation, whereas in the control there is a definite CO_2 deficit throughout the whole test, which here may be attributed to the nitrate assimilation and other synthetic processes just as was the case with

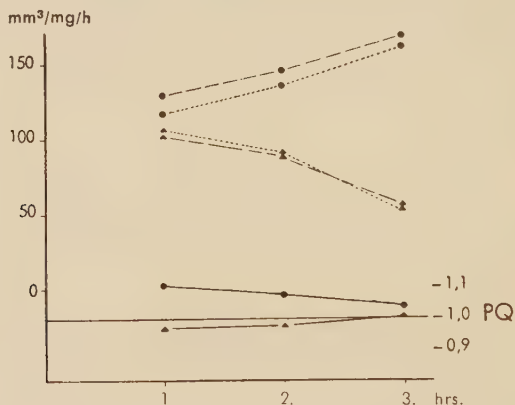


Fig. 26: Effect of an addition of "Systox" on the apparent photosynthesis of *Ankistrodesmus*.

- ● Control
- ▲ ▲ Algae in 0.01 % "Systox" solution
- — Photosynthetic quotients
- - - O_2 production
- CO_2 consumption

Suspension density: 1.0 mg/6 ml. Age of culture: 7 days. Vessel difference method.

the extra carbon dioxide in the corresponding respiration experiments. The slight reduction of the quotient in the control may be due to photooxidative processes resulting from excessively strong illumination. The experiment serves to confirm the results of the other photosynthetic measurements, and at the same time also supplements the findings of the RQ determinations.

F. Appendix: Field tests on *Vicia faba* L.

The tests described here have been concerned chiefly with the nature and importance of disturbances which may occur when "Systox" and "Schradan" preparations are applied in excessive dosages. At the same time it was revealed that a dosage of medium or low concentration did not cause even the slightest disturbance on the examined objects.

* For the application of the vessel difference method, vessels were used with a total volume of 22 and 32 ml, and a liquid volume of 8 ml.

It was mentioned in the introduction that the impression has occasionally been gained in agricultural circles that the preparation "Systox" is not only extremely compatible with plants at the concentrations applied in the field but also that the lowest dosages also have a "stimulating" effect on the growth of the plants. Although the described experiments did not give any indication of the existence of such an effect exerted either by "Systox" or "Schradan", it was, however, decided to take up this question once again by conducting a field test under outdoor conditions.

In the field test, it is practically impossible to completely rule out a slight pest infestation which, under certain circumstances, may even appear insignificant. Therefore, whenever the preparation being tested is applied this pest fauna, even though small, is eliminated. Consequently there will be an increased yield in the treated plots, and in most cases it will not be possible to decide with certainty whether this increase is the result of the insecticidal effect or is due to a physiological effect on metabolism exerted by the applied preparation.

Nicotine plot

		O ₁		F ₁		
O ₂	F ₂					
				O ₃	F ₃	
	F ₄				O ₄	

"Systox" plot

G ₄	O ₄	G ₃	D ₃	E ₂	G ₁	A ₁	O ₁
E ₄	A ₄	E ₃	B ₃	C ₂	F ₂	O ₂	B ₁
C ₄	D ₄	F ₂	O ₃	A ₂	G ₂	G ₁	D ₁
B ₄	F ₄	C ₃	A ₃	D ₂	B ₂	E ₁	F ₁

Fig. 27: Distribution of plots in the outdoor spray test on *Vicia faba*.

In view of the nature of the problem with which we are faced here, it is, therefore, of advantage when the pest infestation in the test plots is as slight as possible. The weather during spring and summer of 1953, in contrast to the previous year, was extremely moist and cool. It was expected that these weather conditions, in contrast to those of 1952, would almost completely prevent the occurrence of aphids. Fig. 28 gives details of the weather conditions during the spring and summer of 1953 at the Research Station, Höfchen. The averages for the nine years previous to 1953 are also given for comparative purposes*.

Vicia faba L. was selected as the test object for this experiment. Outdoor test controls had already been carried out in 1952 at Höfchen using "Systox" against *Doralis fabae* occurring on *Vicia faba* (Unterstenhöfer 1953).

The aphid infestation on *Vicia faba* at the Höfchen station was so low in 1953 that on July 30 of that year a definite aphid colony was found only on every 48 plants (± 8 , with 7 counts). Some influence of the preparation on the plants, in addition to the insecticidal effect, should have made itself noticeable under these conditions. The arrangement of the test is illustrated in Fig. 27.

* Data taken from the records kept by the meteorological section of the Research Station, Höfchen.

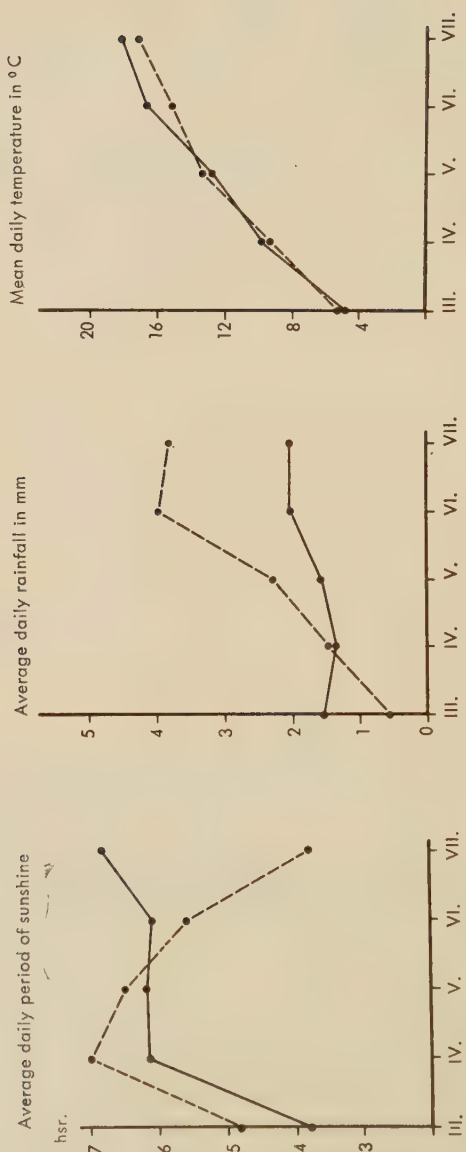


Fig. 28: Weather conditions from March to July at the Research Station Höfchen
 — Average values for the period 1943 — 1952
 - - - Values for 1953

The beans ("Weisskeimige Zwyndrechter" variety) were sown on March 9, 1953 with a spacing of 50×40 cm between each. Two seeds were sown in each plant hole. The whole test field was divided up into plots each measuring 4×5 metres.

In addition to the "Systox" treatment, several of the plots were treated with a nicotine preparation. Slight stimulation of growth, which would be explained by the eradication of several secondary pests and by the extermination of the few aphids present should, therefore, have been effected also in the nicotine plots.

However, if "Systox" were to act here on the processes of metabolism in a positive sense, then it would have been expected that there would be increased yields in the "Systox" plots, in fact yields which would be much larger than any likely increases in the nicotine-treated plots.

In addition to the spray treatment, the seed was also dressed in one test series. The seed was treated before sowing with a powder preparation of "Systox" and active charcoal. All the tests were repeated four times. The entire experiment was divided up into the following series:

- O, A, B (1—4) Control
- C (1—4) 0.05 %, 1 $\times$ sprayed
- D (1—4) 0.10 %, 1 $\times$ sprayed
- E (1—4) 0.05 %, 2 $\times$ sprayed
- F (1—4) 0.10 %, 2 $\times$ sprayed
- G (1—4) dressed

The sprays were carried out on the following dates: E, F: May 29, 1953; C, D, E, F: June 16, 1953. No particular plan was used for dividing up the plots in the test field.

The young plants were fairly heavily infested by *Sitones lineata* at an early stage. The infestation, however, soon receded to such an extent that there were hardly any noticeable injuries by the time the blossom opened. There was only a very slight occurrence of *Apion pisi*. Reference has already been made to the slight aphid infestation. The infestation did not increase at all from the time of the last count until the date of the harvest (July 8 and 9, 1953). The stand was weakly infected all over by mosaic virus of beans.

Table 10 gives a summary of the harvest results obtained in this experiment. In addition to the yields of the individual plots ($\frac{1}{2}$ kg), the average yields are also given in dz (Doppelzentner = 100 kg) per hectare (= 2.47 acres). The errors for the average values are also given in percentages. The yields in the nicotine test, and also those in the control, are well below the corresponding yields of the "Systox" test. These differences may be explained by the fact that the nicotine test was carried out on a field 50 metres away from the "Systox" test, where there was a slightly different gradient, and where the light conditions were different from those in the "Systox" plots. It was, therefore, to be expected that the two tests as such would produce comparable results, but that they could not be compared exactly with each other from the quantitative aspect.

The yields of legumes and beans and the yields of tops and roots were determined to evaluate the experiment. Since it was not possible to remove the tops so carefully in plots as the legumes, and also because a slight, varying percentage of adhering soil was weighed together with the roots, the standard

Table 10: Harvest results obtained in a field test with *Vicia faba* L. The individual plots were treated with different concentrations of "Systox" and nicotine in accordance with the spraying plan given on Page 311.

Harvest results in "Systox" plots																	Harvest results in nicotine plots							
Legumes and Beans									Tops and Roots								Legumes and Beans				Tops and Roots			
O	A	B	C	D	E	F	G	O	A	B	C	D	E	F	G	O	F	O	F	O	F			
1	49	46	50	51	49	52	50	52	72	61	72	96	65	77	79	78	54	52	102	87				
2 $\frac{1}{2}$ kg	51	47	49	51	52	60	49	65	75	70	81	71	70	81	75	85	51	54	104	100				
3	48	58	48	53	47	52	52	45	73	77	91	85	95	72	78	85	56	57	109	110				
4	45	44	42	44	53	43	49	33	90	58	86	86	74	73	77	84	54	60	99	92				
average values $\frac{1}{2}$ kg	48	49	47	50	50	52	53	49	78	67	83	85	76	76	77	84	53	55	104	97				
d/ha	120	122	117	125	125	130	131	122	196	167	207	212	189	189	192	210	133	137	260	242				
standard deviation of mean	1.1	1.8	1.3	1.4	1.1	1.05	1.4	2.6	4.2	4.3	4.1	5.2	2.1	2.1	0.9	1.8	1.1	1.8	2.1	5.0				
standard deviation of mean η_0	2.3	3.7	2.8	2.8	2.2	2.0	2.6	5.3	5.4	6.4	5.0	6.1	2.8	2.8	1.2	2.1	2.1	3.3	2.0	5.1				

deviations of means for the yields of tops and roots are slightly higher than the corresponding values for beans.

Table 10 shows that the root, shoot and leaf yields in the "Systox" and in the nicotine test did not increase at all above the yields of the control plots either as the result of spraying or as the result of the seed treatment. All the differences are within the limits of error and, moreover, there is no tendency for them to be uniform.

The yields of legumes increase slightly as the "Systox" treatment is intensified. The plots sprayed once have a very slightly better yield than the controls, and the 2nd spray also increases the yields by a small amount. On the other hand, the variation of the concentrations does not have any noticeable effect here.

However, when these slight yield increases are compared with the standard deviations of the means, then it is found that in this test the application of "Systox" did not produce any yield increases whatsoever which can be determined statistically by means of the usual method of calculation. Moreover, a comparison with the nicotine tests shows that here, too, there is a certain difference between the control and the treated plots which apparently must be attributed to the success of the control against the few pests which occurred in the plots.

In summarizing, it may be said that in these fields tests, with an almost complete absence of pest infestation, no yield increases were obtained by spraying broad beans (*Vicia faba* L.) with "Systox". These results completely justify the view gained in the laboratory test that the ester examined here, apart from its insecticidal potency, is not able to improve the balance of metabolism of the plants, nor the yields, to any measurable extent.

IV. Discussion of the results

A survey of the tests shows that no injuries whatsoever are to be expected when "Systox" of low and medium concentrations (0.0001 to 0.001 %) is applied direct to the different types of plants examined here. It is not until stronger concentrations are applied that different forms of disturbances are caused. When this finding is compared with the results of animal physiological experiments, it is observed that the animal objects are considerably more sensitive. Mosquito larvae, for example, are killed even in a "Systox" solution of only 0.00001 % (approx. 0.00000043 molar).

With respect to warm-blooded animals, it is generally maintained that the poisoning symptoms caused by preparations of the phosphoric ester group are to be attributed primarily to an inhibition of the cholinesterase in the region of the motor end plate. According to Duspiva, it appears that allowance will have to be made for a central significance of the cholinesterase inhibition following poisoning by phosphoric esters also with insects, despite the findings of Tobias et al., Hopf etc. It has not been completely clarified to what extent other disturbances of the physiological processes, apart from the inhibition of the cholinesterase, can be attributed direct to the toxic effect of phosphoric esters.

In protozoa, for which a significance of the cholinesterase has not been established, disturbances of movement and reaction first occur above a concentration of 0.001 % "Systox" in the solution (verbal report of Hecht). There is no evidence available of cell-physiological observations having been made on these objects after disturbances have set in which are caused by ester preparations.

The toxic effect of phosphoric esters on insects and warm-blooded animals is, therefore, apparently a specific, centrally attacking inhibition of the enzyme. On the other hand, another mechanism of toxic action will have to be found to explain the toxic action of those preparations which do not become effective until stronger concentrations are applied on objects in which the acetylcholin-cholinesterase system, or a closely related mechanism does not have such a central location, particularly in plant cells.

Reference should be made, however, to a finding of Aldridge which is of particular interest in this respect. He reports that acetylcholinesterase isolated from plant tissues (oranges) is inhibited by the effect of organic phosphoric esters (tetraethyl pyrophosphate). This corresponds to the many observations of the inhibition of animal esterases by phosphoric esters (literature; cf. Duspiva).

From studies he made on insects, Jochum arrived at the opinion that the poisoning symptoms caused by "Folidol E 605", irrespective of a cholinesterase inhibition, must be attributed to a primary disturbance of the water equilibrium of the poisoned animals. The reduction in the lymphfraction, regarded as primary, is due to an increase in the permeability to water after the ester has penetrated into the cell surface layers.

These conceptions can hardly be applied direct to the conditions which prevail following the effect of ester preparations on plant tissues, even though the assumption that the permeability to water is increased might provide a simple explanation for the finding of Tietz that the absorption of water by roots may be increased by "Systox" dosages. Reference should also be made to the finding of Linskens (1952) who reports that "Folidol E 605" has the effect of stimulating the swelling of lupin and sinapis seeds.

In the tests described in this paper, which primarily are concerned more with the phytophysiological effect of "Systox", a wide range of general disturbance occurred on the treated objects as soon as the harmful concentration was reached. Observations of *Ankistrodesmus* and *Lemna* showed the cell division processes to be relatively susceptible.

When the preparation is applied in excessive dosages, the values for photosynthesis, nitrate assimilation and other synthetic processes drop below the corresponding values of the preparation-free control in approximately the same concentration range. The oxidative assimilation of glucose remains unaffected at first. The nitrate assimilation is inhibited in light and in darkness, i. e. irrespective of the photosynthetic activity of the cells. This inhibition applies both to the actual nitrate reduction as well as to the syntheses necessary for incorporating the ammonium salt.

The synthetic inhibitions are apparently not so serious as to prevent a further increase of substance. It is, however, to be expected that the chemical composition of the cell will be changed in favour of the carbohydrates. This also explains the occurrence of giant cells in *Ankistrodesmus* following a "Systox" treatment, which is generally observed both with inhibited division as well as following an overdosage of carbohydrate.

An increase in respiration is caused at the same time as the different inhibitions. Judging by previous findings, this effect may be attributed to the presence of rearrangement products or by-products in the preparation. Past

observations (Hartley; March et al.) and results of studies not yet concluded also support the theory that such secondary constituents do occur.

The phytopathological experiments, in contrast to the animal physiological studies, therefore, suggest a really general action on the part of the preparation. The simultaneous effects exerted on the various processes of metabolism indicate that the mechanism of toxic action is non-specific. This basic difference in the effect of "Systox" on animal and plant objects is the reason for the large gap between the *dosis curativa* and the *dosis toxica*, which is one of the most important conditions for the usefulness of the preparation as a systemic insecticide.

When an attempt is made to give a fuller explanation of the phytopathological effects of "Systox", it is possible first of all, according to chemical experience with preparations, to exclude the possibility of a heavy metal-complex formation causing the inhibition of a number of enzyme systems (verbal report of Schrader).

However, when it is considered that the structure of "Systox" is more or less unpolar and that the product has strong lipophile properties (Schrader), then it is to be expected that there will be certain changes in the surface conditions in heterogeneous systems which have lipophile and lipophobe phases. Such changes in surface conditions caused by "Systox" would have a direct bearing upon a number of cell-physiological processes which occur on these surfaces. It is, therefore, quite obvious that a non-specific and general disturbance of the cell functions must result in consequence of this.

The injurious effects discussed here will not occur in practice when the preparation is applied at the normal concentration. From the field tests conducted on *Vicia faba* (Unterstenhöfer 1953), it appears that the concentration limit at which injuries are inflicted, is reached when three treatments are carried out with 0.1 % "Systox". The first injuries are noticeable in this case from the harvest figures but they cannot be guaranteed statistically. Disturbances of the plant metabolism naturally occur earlier when, instead of spraying the leaf of a cultivated plant, the active substance is applied direct to the individual cells under examination, such as was the case in our experiments. A relatively large percentage of the applied quantity of active substance does not reach the plant when the preparation is applied in the field (Tietz). The remainder of the active substance which penetrates into the leaf is considerably diluted there, and the concentration is further reduced by translocation. It was also shown that when the insecticide is applied by the watering technique at the usual dosages, no harm will be caused to the roots.

If there is reason to believe that the total balance of metabolism will not be affected by standard concentrations of "Systox" when the preparation is applied in the normal manner, then attention should also be given to the question as to what extent a stimulation effect may be expected when "Systox" is applied at normal or even low concentrations.

Certain agricultural circles say that the insecticidally effective phosphoric esters also have a stimulating effect (i. e. a growth and yield stimulating effect in accordance with the terminology customary in applied research) (references, see P. 267). Such reports must firstly be checked carefully, and treated with the usual reserve. In addition to the observations that phosphoric esters have the effect of increasing yields, reports have also been made of positive effects being produced following the application of toxaphene (Wille), DDT (Chapman

and Allen, Callinan), hexachlorocyclohexane (Runge, Sellke), wettable sulphur (Hadorn), thiourea (Popzow), sulphurous acid and nitrous gases (Wehner), 2,4,5-T esters (Agr. Res. Anon.), mercury dressings (Gassner), "Captan" (Calif. Spray Chem.) and other substances.

During the course of these experiments, there was no indication either in the laboratory test or outdoors that "Systox" and "Schradan" influence the basic metabolic processes of the examined objects in such a way as to cause an improvement of the total balance of metabolism. When low and medium concentrations are applied, the active substance and emulsifier of "Systox" behave completely indifferently in every case.

The large number of papers reporting on positive effects of phosphoric esters other than the insecticidal effect, however, include cases where the effects are so obvious that they simply cannot be left unconsidered when discussing the effects of "Systox". In a number of instances, the described effects can be attributed simply to indirect or relative stimulation.

Attention is often drawn to dry injuries in the control plots of field tests (Wolfenbarger, Zattler, 1952). As test plants treated with phosphoric ester preparation ("Folidol E 605") enjoy an advantage when there is a lack of water due to an improvement of the wettability (Linskens) (better utilization of dew), then the controls must undoubtedly suffer a yield depression in such cases, which may be incorrectly interpreted as a stimulation of the treated plants. Furthermore, a slight infestation of pests, which may perhaps appear to be of secondary importance, can never be fully eliminated in field tests. Therefore, in many cases the observed yield increases may be interpreted as the result of an effective control of even an insignificant pest fauna leading to slight improvements of the harvest thus giving the impression of a direct positive influence being exerted by the preparation on the metabolic processes of the treated plant.

In closing, it may be said that low and medium dosages of "Systox" have no effect whatsoever on the physiological processes in the plants examined. When the preparation is applied in excessive dosages, starting from a certain concentration limit, a range of injuries with the most general of symptoms can be established for every insecticide. At least, this is how the disturbances mentioned in this paper are to be understood. It is important for the value of an insecticide that this limit is not reached at normal application. The difference between the *dosis curativa* and the *dosis toxica* is so great with "Systox" that this product appears to meet the high standards of phytophysiological compatibility required of a systemic insecticide.

V. Summary of the results

1. The growth of the cress root is inhibited by "Systox" solutions containing a concentration of active substance stronger than 0.005 %. while solutions of a weaker concentration have no influence on growth whatsoever. Comparative studies made on *Lemna* roots suggest that this is primarily an inhibition of the division processes, while at first cell elongation and plasm growth are less affected. The occurrence of giant cells during the cultivation

of the unicellular green alga, *Ankistrodesmus*, in a saturated solution of active substance may be explained in a similar manner.

2. The dry weight production of *Ankistrodesmus* decreases considerably in solutions of 0.005 % "Systox" and 0.014 % "Schradan". Low and medium concentrations have no influence on the growth of the cells.
3. The use of a ^{32}P -labelled "Systox" preparation revealed that algae rich in fat (nitrogen deficiency) store more active ingredient ($> 30\%$) than fat-free cells. It is far more difficult to wash the preparation out of fat-storing cells than out of normal cells.
4. In "Systox" solutions ($> 0.005\%$ content of active substance), there is a reversible increase of the respiratory consumption of O_2 and the production of O_2 (*Ankistrodesmus*, *Chlorella*, *Helodea*). When fresh solutions are used, this disturbance occurs only in the presence of the $\text{P}=\text{S}$ isomer but not in the solutions and emulsions of the $\text{P}=\text{O}$ isomer. Old solutions of the $\text{P}=\text{O}$ isomer also increase the respiration. Weak concentrations of active substance behave indifferently. It is assumed that the increased respiration is due to an effect caused by rearrangement products or by secondary constituents.
5. The increased respiration caused by "Systox" is not amplified by an addition of glucose. The exogenous glucose respiration attains its maximum value in the presence of "Systox" more slowly than in the control. It is possible that a part is played here by changes in permeability. There was no evidence of a change in the synthetic efficiency of glucose in the presence of "Systox".
6. There is no increase of the respiratory consumption of O_2 in *Tradescantia* and sugar beet roots after "Systox" is added. It is not until solutions containing more than 0.06 % active substance are applied that serious irreversible injuries are caused on *Tradescantia* roots. By using a radioactive labelled preparation, it was revealed that when the preparation is applied to the soil by the watering technique in the region of the roots, no concentrations are produced which adversely affect the growth and metabolism of the roots.
7. The concentrations which increase respiration ($> 0.005\%$ active substance content) cause a depression of photosynthesis of about 20 % on *Ankistrodesmus* and *Helodea*. Both isomers act equally on photosynthesis. The same effect was produced by a "Schradan" solution of comparable concentration. Low concentrations also have no effect here. The depression of the photosynthetic efficiency can be remedied much more quickly by removing the preparation, than the increase of respiration.
8. The syntheses which are involved with the incorporation of ammonium salt, and the nitrate assimilation are inhibited in a 0.01 % "Systox" solution. The respiratory and photosynthetic quotients are consequently much lower in ammonium and nitrate-containing nutrient solutions in the presence of "Systox" than the corresponding values in the preparation-free control.
9. Field tests conducted on *Vicia faba* showed that an increase in the yield is not to be expected following an application of "Systox" even when pest infestation is completely eliminated. This finding fully corresponds with the results of the laboratory test.

VI. Appendix : Formulae

Formulae for correcting the results obtained with the Geiger-Müller counter

1. Errors arising out of poor selectivity of the measuring apparatus are eliminated by applying the following formula:

$$\text{The true impulse value } N_0 = \frac{N}{1 - N\tau}$$

N is the number of impulses registered by the apparatus. τ is the resolving time in seconds, the time which must elapse between 2 impulses when they are to be registered separately. τ was quoted at 10^{-5} for the apparatus used.

2. The zero effect measured in the same time should be subtracted from the determined value N_0 .
3. After making these two corrections, the determined value must be converted to day 0, any time to be selected after the production of the isotope, because the activity of the preparation decreases as time (in days) proceeds. The activity decrease depends upon the disintegration constant λ .

$$\text{True impulse value on day 0:} \quad N = N_0 \cdot e^{\lambda t}$$

When T is expressed as the function of the half-life value, then

$$N = N_0 \cdot e^{-(1.386) \frac{t}{T}}$$

Therefore, for ^{32}P with a half-life value of 14.295 days, this correction factor amounts to

$$N = N_0 \cdot e^{(-0.0485 \quad t)}$$

Manometric method Formulae for calculating the gas metabolism

$$\text{Vessel constant} \quad K = \frac{V_G \frac{273}{T} + V_F \alpha}{P_0}$$

Key:

V_G : Volumes of gas in mm^3

V_F : Liquid volumes in mm^3

T : Absolute test temperature

α : Bunsen's absorption coefficient of the measured gases (CO_2 and O_2)

P_0 : Normal pressure in mm Brodie solution (10,000)

I. Direct KOH method

$$x_{O_2} = h k_{O_2}$$

x = Quantity of gas in mm^3 produced or consumed during the test period (760 mm, 0°C)

h = Observed change of pressure in mm *Brodie* solution

k = Vessel constant

2. Direct two gas method

$$x_{O_2} = h \cdot \frac{k_{O_2} \cdot k_{CO_2}}{k_{CO_2} + \gamma k_{O_2}}$$

$$\gamma = \text{gas metabolism quotient} = \frac{+ x \text{ CO}_2}{- x \text{ O}_2}$$

3. Vessel difference method

$$x_{O_2} = \frac{h k_{CO_2} - H K_{CO_2}}{\frac{k_{CO_2}}{k_{O_2}} - \frac{K_{CO_2}}{K_{O_2}}}$$

$$x_{CO_2} = \frac{h k_{O_2} - H K_{O_2}}{\frac{k_{O_2}}{k_{CO_2}} - \frac{K_{O_2}}{K_{CO_2}}}$$

H : Pressure change in vessels with small gas volumes

h : Pressure change in vessels with large gas volumes

K : Vessel constant of vessels with small volumes

k : Vessel constant of vessels with large volumes

4. $\pm$ KOH method

Determination of x_{O_2} according to the direct KOH method:

$$x_{O_2} = h k_{O_2}$$

$$x_{CO_2} = (h' - \frac{x_{O_2}}{k'_{O_2}}) k'_{CO_2}$$

h' and k' : Values for the vessels without KOH

References

Alberts-Dietert, F., 1941:

„Die Wirkung von Eisen und Mangan auf die Stickstoffassimilation von *Chlorella*“; *Planta* 32, P. 88—117.

Aldridge, W. N., 1954:

“Anticholinesterases, Inhibition of Cholinesterases by Organo-Phosphorus Compounds and Reversal of this Reaction”, *Chem. and Ind.* 17, P. 473—476.

Allen, T. C., and J. E. Casida, 1951:

“Criteria of Evaluating Insecticidal Phytotoxicity — Areal Growth”; *Journ. Econ. Ent.* 44, P. 737—740.

- Ashdown, D., and H. B. Corder, 1952:
 "Some Effects on Insect Control and Plant Response of a Systemic Insecticide Applied as a Spray, a Seed Treatment or a Soil Treatment"; Journ. Econ. Ent. 45, P. 302—307.
- Beck, H., 1952:
 „Über die Wirkung verschiedener neuer Kontaktinsektizide auf das Wachstum der Kressewurzel“; Zeitschr. f. Pflanzenbau und Pflanzenschutz 5, P. 222—238.
- Bosse, H. C., 1952:
 „Versuche mit einem neuartigen Schädlingsbekämpfungsmittel im Gartenbau“; Höfchen-Briefe 5, P. 81—88.
- Brüggemann, O., 1954:
 „Wirkungen des Stickstoffmangels bei einzelligen Grünalgen“; Diss. Marburg.
- California Spray Chem. (Anonymous), 1954:
 „Zweite europäische Orthocidkonferenz“; Zürich, Nov. 1954; Calif. Spray Chem. Comp. Francaise.
- Callinan, F. P., 1949:
 "New Insecticides, their Effects on Plants and Soil"; Journ. Ent. 42, P. 387—391.
- Casida, J. E., and T. C. Allen, 1951:
 "A Laboratory Method for Evaluating the Phytotoxicity or Phytostimulation of Insecticides"; Science 113 (2941), P. 553—555.
- Casida, J. E., and T. C. Allen, 1951:
 "Criteria for Evaluating Insecticidal Phytotoxicity — Root Growth"; Journ. Econ. Ent. 44, P. 741—746.
- Casida, J. E., and R. K. Chapman and T. C. Allen, 1952:
 "Reaction of Absorption and Metabolism of Octamethylpyrophosphoramid by Pea Plants to Available Phosphorus", Journ. Econ. Ent. 45, P. 568—578.
- Chapman, R. K., and T. C. Allen, 1948:
 "Stimulation and Suppression of some Vegetable Plants by DDT"; Journ. Econ. Ent. 41, P. 616—623.
- Chodat, R., 1909:
 « Étude critique et expérimental sur le polymorphisme des Algues »; Genève.
- Ciferri, E., and Bertossi, F., 1949 (Zit. Beck):
 Experientia 5, P. 280—281.
- Clauss, H., 1952:
 „Kritische Untersuchungen über den Kressewurzeltest von Moewus“; Z. Naturf. 7b, P. 112—117.
- Cox, A. J., 1943:
 "Terminology of Insecticides, Fungicides and other Economic Poisons"; Journ. Econ. Ent. 36, P. 813—821.
- Cramer, M., and J. Myers, 1949:
 "Effects of Starvation on the Metabolism of Chlorella"; Plant Physiol. 24 P. 255—264.
- David, W. A. L., 1951:
 "Insecticidal-action studies with bis(dimethylamino)phosphorous anhydride containing ^{32}P Phosphorus"; An. appl. Biol. 38, P. 508—524.
- Dixon, M., 1951:
 "Manometric Methods" (3rd Edition); Cambridge University Press.
- Duspiva, F., 1954:
 „Untersuchungen über die Wirkungsweise von Insektiziden“; Verh. d. deutsch. Ges. f. angew. Entom. (12. Mitgl.-Vers.); P. 129—134.
- Emerson, R., and L. Green, 1938:
 "Effect of hydrogen ion concentration on Chlorella Photosynthesis", Plant Physiology 13, P. 157—168.
- Endrigkeit, A., 1954:
 „Ist eine weitere Vereinfachung der Kohlfiegenbekämpfung möglich?“; Gesunde Pflanze 6, P. 145—150.
- Foster, A. C., 1951:
 "Some Plant Responses to certain Insecticides in the Soil"; U. S. Dept. agric. Circ. 862, P. 41.

- Frohberger, P. E., 1949:
„Untersuchungen über das Verhalten des Insektizides Diäthyl-p-nitrophenylthiophosphat (E 605') auf und in der Pflanze“, Höfchen-Briefe 1949/2, P. 10–91.
- Gaffron, H., 1939:
„Über auffallende Unterschiede in der Physiologie nahe verwandter Algenstämme, nebst Bemerkungen über Lichtatmung“, Biol. Zentralbl. 59, P. 302–313.
- Gassner, G., 1953:
„Frühtreiben durch Beizung mit quecksilberhaltigen Beizmitteln“, Angew. Bot. 27, 2. P. 37–47.
- Gassner, R.:
„Über das Verhalten von selektiven Insektiziden mit Tiefenwirkung in der Pflanze“, Ber. d. Schweiz. Botan. Gesell. 62, 1952, P. 66–79.
- Geisler, E., 1950:
„Einige Beobachtungen über den Einfluß des Hexachlorcyclohexan auf die Pflanze“, Nachrichtenbl. f. d. dt. Pflanzenschd. 2, P. 131–135.
- Göllner, E., 1952:
„Untersuchungen zur Zellphysiologie des Stickstoff- und Phosphormangels grüner Pflanzen“, Diss. Marburg.
- Hadorn, C., 1952:
„Die biologischen und chemischen Grundlagen der Schädlingsbekämpfung“, Der Pflanzenarzt, Vol. 5, March 1952.
- Hanna, A. D., 1952:
„Investigations on the Use of Systemic Insecticides to Control the Mealybug Vector of the Swollen Shoot Disease in Cocoa“, Proc. 3rd Inter. Congr. Crop Prot., Paris.
- Hartley, G. S., 1952:
„The Anomaly of 'Systox'“, World Crops, November, P. 397.
- Hecht, G., and W. Wirth, 1950:
„Zur Pharmakologie der Phosphorsäureester“, Arch. exp. Path. u. Pharmacol., Vol. 211, 3, P. 264–277.
- Hopf, H. S., 1952:
Ann. Appl. Biol. 39, P. 193 (Zit. Duspiva).
- Hopf, H. S., and W. R. Boom, 1951:
„Injection Experiments on Cholinesterase Inhibition of Insecticides on Locusts“, Abstr. Paper IXth Int. Congr. Ent. Amsterdam IV, 5.
- Jochum, K. F., 1954:
„Durch Diäthyl-p-Nitrophenylthiophosphat abgeänderte Reaktionsketten im Insektenorganismus“, Diss. Köln.
- Incho, H. H., and Greenby, 1952:
„Synergistic Effect of Piperonylbutoxyde with the Active Principles of Pyrethrum and with Allethrolone-esters of *Chrysanthemum* acids“, Journ. Econ. Ent. 45, 5, P. 794–799.
- Kasting, R., and Woodward, J. C., 1951:
„Persistence and Toxicity of Parathion when added to the Soil“, Sci. Agric. 31, P. 133–138.
- Kessler, E., 1953:
„Über den Mechanismus der Nitratreduktion von Grünalgen“, Flora od. Allgem. Botan.-Ztg., Vol. 140.
- Kessler, E., 1953:
„Über den Mechanismus der Nitratreduktion von Grünalgen, II“, Arch. f. Mikrobiol., Vol. 19, P. 438–457.
- Krebs, H. A., 1929:
„Methoden der manometrischen Messung von Atmung und Gärung“ in Oppenheimer „Die Fermente“, Vol. 3, P. 646 ff.
- Krollpfeiffer, J., 1951:
„Untersuchung der Gaswechselquotienten von Grünalgen und deren Beziehung zur Nitrataassimilation“, Diss. Marburg.
- Linskens, H. F., 1952:
„Über den Einfluß von 'E 605' auf den Quellungsverlauf“, Höfchen-Briefe 5, 1, P. 33–35.

- Linskens, H. F., 1951:
„Über Änderung der Benetzbarkeit von Blattoberflächen nach Spritzungen“;
Ztschr. f. Pflanzenkrankh. und Pflanzenschutz, Vol. 58; P. 327—322.
- March, R. B., Metcalf, R. L., and Fucuto, T. R., 1954:
“Paper chromatography of the systemic insecticides Demeton and ‘Schradan’”;
Agric. and Food Chem. 2, 14, P. 732—735.
- Martin, H., 1947:
“Important new Discoveries in Plant Protection; Grower, April, P. 26.
- Martin, R. P., and Russel, R. S., 1950:
“Studies with Radioactive Tracers in Plant Nutrition II”; Journ. exp. Bot. 1,
P. 141—158.
- Mayer, E. L., McGovran, E. R., Tallay, F. B., and Willaman, J. J., 1950:
“Tests for Synergism between Nicotine and Phtalonitrile and between Nicotine
and 2,3,4,5,6-Pentachloranisole”; Journ. Econ. Ent. 43, P. 533—537.
- Moewus, F., 1949:
„Der Kressewurzeltest, ein neuer quantitativer Wuchsstofftest“; Biol. Zentral-
bl. 68, P. 118—140.
- Moewus, F., 1948:
„Ein neuer quantitativer Test für pflanzliche Wuchsstoffe“; Naturwiss. 35,
P. 124—125.
- Müller, A., 1926:
„Die innere Therapie der Pflanze“; Berlin.
- Myers, J., 1949:
“The Pattern of Photosynthesis in *Chlorella*” in: “Photosynthesis in Plants”;
Edited by J. Franck and W. E. Loomis, The Iowa State Coll. Press, P. 349—364.
- Myers, J., 1947:
“Oxidative Assimilation in relation to Photosynthesis in *Chlorella*”; J. Gen.
Physiol. 19, P. 217.
- Niemann, E., 1952:
„Vergleichende Untersuchungen über die Ausscheidung keimungshemmender
Stoffe aus Früchten und Samen unter besonderer Berücksichtigung von *Foeni-
culum vulgare* Miller“; Flora 139, P. 195—199.
- Nolan, K., and Wilcoxon, F., 1950:
“Method for Bioassay for Traces of parathion in Plant Material”; Agric. Chem.
5 (1), P. 53—74.
- Pirson, A., and Göllner, E., 1953:
„Beobachtungen zur Entwicklungsphysiologie der *Lemna minor* L.“; Flora,
Vol. 140, P. 485—498.
- Pirson, A., and Kellner, K., 1952:
„Physiologische Wirkungen des Rubidiums“, Ber. Dtsch. Bot. Ges., Vol. 65, 8,
A. 276—296.
- Pirson, A., Krollpfeiffer, I., and Schäfer, G., 1953:
„Leistungsfähigkeit und Fehlerquellen manometrischer Stoffwechselfmessungen“,
Marburger Sitzungsberichte, 76, No. 2, P. 3—27.
- Pirson, A., and Seidel, F., 1950:
„Zell- und stoffwechselphysiologische Untersuchungen an *Lemna minor* L.“;
Planta, Vol. 38, P. 431—473.
- Pirson, A., Tichy, C., and Wilhelmi, G., 1952:
„Stoffwechsel und Mineralsalzernährung einzelliger Grünalgen“; Planta, Vol. 40,
P. 199—253.
- Popzow, A. W., 1952:
„Thioharnstoff als Stimulans beim Keimen der Samen von Kok-Saghyes und
Tau-Saghyes“; Ber. d. Akad. d. Wiss. d. UdSSR (neue Serie), Vol. 84, 3,
P. 619—622.
- Fringsheim, E. G., 1951:
„Die Sammlung von Algen-, Flagellaten- und Mooskulturen am Botan. Inst.
der Universität Cambridge“; Arch. f. Mikrobiol. Vol. 16, P. 1—17.
- Reinert, A. W., 1952:
„Wuchsstoffteste“; Ztschr. f. Bot. Vol. 40, 1, P. 77—81.

- Ripper, W. E., Greenslade R. M., and Lickerish, L. A., 1949:
 "Combinated Chemical and Biological Control of Insects by Means of a Systemic Insecticide"; *Nature* 163, P. 787—789.
- Rümker, v. R., 1951:
 „Über die Ökologie von *Askochyta pinodella* und *Fusarium culmorum* in der Rhizosphäre anfälliger und nicht anfälliger Pflanzen“; *Phytopath. Ztschr.*, Vol. 18, 1.
- Runge, N., 1952:
 „Ertragssteigerungen bei Tomaten und Bohnen, bedingt durch eine Blüten-spritzung mit Hexachlorcyclohexan“; *Angew. Bot.*, Vol. 26, 3/4, P. 130—138.
- Russel, R., and Martin, R. P., 1950:
 „Studies with Radioactive Tracers in Plant Nutrition I“; *J. exp. Bot.* 1, P. 133—140.
- Schopfer, W. H., and Bein, M. L.:
Experientia 4, 1948, P. 147—148 (Zit. Beck).
- Schrader, G., 1952:
 „Die Entwicklung neuer Insektizide auf Grundlage organischer Fluor- und Phosphorverbindungen“; *Monogr. z. Angew. Chem. und Chem. Ing. Techn.* 62.
- Schubert, G., Dittrich, W., Künkel, H., and Schwermund H. J., 1951:
 „Bedeutung und Anwendung der Isotopenforschung in Biologie und Medizin I“, *Strahlentherapie* 84, P. 165, II. *Strahlentherapie* 84, P. 328.
- Schulze, K., 1953:
 „Ungiftige Schädlingsbekämpfung mit natürlichen und synthetischen Pyrethrin-“; *Schädlingsbek.* 5, P. 69—73.
- Sellke, K.:
 „Die Pflanzenschutzmittel der DDR“; *Die deutsche Landwirtschaft.* 27.
- Spoehr, H. A., Smith, J. H. C., Strain, H. H., Millner, H. W., and Hardin, G. J., 1949:
 „Fatty-acid Antibacterials from Plants“; *Carnegie Inst. Washington Publ.* No. 586, P. 67.
- Syrett, P. J., 1951:
 „The Effect of Cyanide on the Respiration and the Oxidative Assimilation of Glucose by *Chlorella vulgaris*“; *Ann. of Bots. N. S.* Vol. XV, P. 473—492.
- Tietz, H., 1953:
 „Das Eindringungsvermögen, die Wanderung und die Ausscheidung von ³²P-markiertem ‚Systox‘ bei höheren Pflanzen“; *Diss. Köln.*
- Tobias, J. M., Kollros, J. J., and Savit, J., 1946:
J. Cell. Comp. Physiol. 28, P. 159 (Zit. Duspiva).
- Turner, N., 1953:
 „Methylenedioxyphenol Synergists for Insecticides“; *The Connecticut Agric. Exper. Station, New Haven, Bull.* 570, April.
- Umbreit, W. W., Burris, R. H., and Stauffer, J. F., 1945:
 „Manometric techniques and related Methods for the Study of Tissue Metabolism“; *Minneapolis, Burgess Publ. Comp.*
- Unterstenhöfer G., 1953:
 „Freilandversuche mit dem systemischen Insektizid ‚Systox‘ gegen die schwarze Bohnenlaus (*Doralis fabae* Scop.) an Ackerbohne (*Vicia faba* L.)“; *Höfchen-Briefe* 1953/2, P. 121—130.
- Unterstenhöfer, G., 1952:
 „Über das innertherapeutische Insektizid ‚Systox‘“; *Meded. Landbouwhogeschool Gent*, 17, P. 75.
- Vischer, W., 1920:
 « Sur le polymorphisme de l'*Ankistrodesmus Braunii* (Naegeli) Collins »; *Ztschr. f. Hydrologie (Aarau)* 1, P. 5—50.
- Warburg, O., and Burk, D., 1950:
 „The maximum Efficiency in Photosynthesis“; *Arch. Biochem.* Vol. 25, P. 423.
- Warburg, O., and Negelein, E., 1920:
 „Über die Reduktion der Salpetersäure in grünen Zellen“; *Biochem. Z.* 110, P. 66—115.

- Wehner, O., 1928:
„Untersuchungen über die chemische Beeinflußbarkeit des Assimilationsapparats“; Planta 6, P. 543—590.
- Wille, J. E., 1952:
„Probleme der landwirtschaftlichen Schädlingsbekämpfung in Peru“; Naturwiss. 39, 5, P. 105—106.
- Wolfenbarger, D. O., 1948:
“Nutritional value of phosphoric Insecticides”; Journ. Econ. Ent. 41, 5; P. 818—819.
- Zattler, F., 1952:
„Innertherapeutische Bekämpfung der roten Spinnmilbe und Blattlaus im Hopfenbau“; Hopfenrundschaue 10, May 1952.
- Zattler, F., 1951:
„Versuche mit ‚Systox‘ und anderen innertherapeutischen Mitteln gegen rote Spinnmilbe und Blattläuse bei Hopfen“; Höfchen-Briefe 1951/4.
- Zattler, F., and Linke, W., 1950:
„Spritzversuche mit Kupfermitteln im Hopfenbau mit und ohne Beimischungen von ‚E 605‘ im Jahre 1949“; Pflanzenbau und Pflanzensch. 2, P. 49—63.
- Zeid, M. M. I., and Kuthomp, L. K., 1951:
“Effects associated with Toxicity and Plant translocation of three Phosphate Insecticides”; Journ. Ent. 44, 6.
- Anonymous, 1953:
“Making Limas Live Longer, Yield More”; Agric. Res. November 1953, 12.